A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS [1,2]

III. HEMOGLOBIN LEVELS OBSERVED IN WHITE RATS WHICH WERE FED TOXIC WHEAT

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THREE FIGURES

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Franke ('34) has reported the occurrence of an apparently new plant toxicant in various cereal grains as well as native grasses. The toxic factor in the plants is related to the nature of the soil and is endemic to particular localities. The affected plants produce very pronounced effects in livestock and also in experimental animals. Franke showed that of 325 rats placed on lethal samples of grain, 299 had died by the hundredth day. It was observed that many of the rats had an anemic appearance at death.

The present investigation was begun in an attempt to classify the type of anemia encountered and to determine its relation to anemias caused by other toxicants. Preliminary investigations showed that extremely low red cell counts might be expected with correspondingly low red cell volumes and hemoglobin levels. Red cell counts as low as 450,000 cells

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[2] These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics, of the United States Department of Agriculture.

per cubic millimeter were observed in moribund animals. Blood smears showed that the erythrocytes were very abnormal, with marked anisocytosis and polychromatophilia. The differential leucocyte count revealed marked irregularities in the ratios between the various white cells. Extremely icteric plasmas were found in a large number of affected animals.

Before beginning a complete hematological investigation, it seemed advisable to determine the incidence of the anemia as well as its severity. The hemoglobin estimation was chosen for this purpose, since it gives a very good measure of the degree of anemia present, and requires a minimum of apparatus and time.

Although the toxicant is known to be present in various affected grains, the hematological investigations have been conducted with wheat only.

PLAN OF EXPERIMENT

The experimental work was done in two stages. The first step was to determine the hemoglobin levels in moribund rats without previous bleeding (series 85). The majority of the animals were sacrificed when they had become too weak to maintain their equilibrium. Rapid slowing of the respiratory rate was also taken as a criterion of approaching death.

The second step was to determine the rate at which the anemia progressed (series 87). Hemoglobin was determined on tail blood at 3-day intervals.

EXPERIMENTAL

The rats used were of Wistar Institute origin. They had been weaned at 21 days and maintained for 1 week on McCollum's Diet I, as described by Burr and Burr ('29). They were then divided into three groups and placed in the individual 'drawer-cages' described by Franke and Franke ('34).

Since the rats receiving the affected wheat diet voluntarily restricted their food consumption to a marked degree, it was

necessary to use a system of double controls, somewhat similar to that described by Swanson and Smith ('32 b). This will be described in detail for series 85.

One group of rats was given the control wheat diet ad lib. These rats served as full controls (group I). Daily food consumption was recorded to the nearest gram. Group II received the diet containing the affected wheat ad lib, and will be referred to as the experimental group. Their daily food consumption was recorded to the nearest tenth of a gram. The rats in group III received the control diet, but their food intake was restricted to the average food consumption of the rats in the experimental group. These rats will be referred to as the restricted controls. It was soon observed that the growth curves of the rats in groups II and III are not strictly comparable due to the wide variations in individual food consumption. Therefore, after the tenth day, each rat in group III received the amount eaten by the corresponding rat in the experimental group. All of the rats were weighed at 5-day intervals until sacrificed. There were ten rats in each group.

Series 87 was organized very much like series 85. The rats were divided into three groups of nine rats each as above. For each experimental rat there was a full control and a restricted control of the same sex, from the same litter, and with approximately the same weight. Food consumption was recorded as in series 85. Each rat in group III received the amount of diet taken by the corresponding experimental rat. Inasmuch as the restricted controls ate their entire quota of diet within 20 minutes after being fed, their hemoglobin values represent virtually 24-hour fasting levels. The rats were weighed and bled at 3-day intervals.

The rats in each of the three groups in series 87 were divided into three sub-groups. Nine rats were bled and weighed every day (three controls, three restricted controls, and three experimental). Since each group contained nine rats, it was possible to bleed each sub-group every third day. By this procedure, individual rats were bled at 3-day intervals, but a continuous daily record of hemoglobin levels for the series could be obtained (fig. 3, which follows).

The high grain diet (diet no. 3) described by Franke ('34) has been modified slightly for the present investigation, and will be given here:

	Per cent
Ground whole wheat	82.0
Commercial casein	10.0
McCollum's salt mixture no. 185	1.0
Pure leaf lard	3.0
Dehydrated yeast (Northwestern)	2.0
Cod liver oil	2.0

The control diet was made with wheat which was considered 'normal,' and the experimental diet was made with wheat (lab. no. 582) classified as lethal (i.e., producing death in the majority of cases by the sixtieth day). This diet gives excellent growth when made with normal wheat.

Fresh distilled water containing a trace of iodine was available to the rats at all times.

Hemoglobin was determined by the improved Newcomer method, using a Bausch & Lomb standard disc, blue color filter, and colorimeter. Although the authors did not restandardize the hemoglobin disc, it was felt that the figures obtained would be relatively correct, notwithstanding possible error on the part of the manufacturer. In series 85, the blood was withdrawn from the abdominal aorta by means of a hypodermic needle and syringe, according to Swanson and Smith ('32 a). In series 87, blood was obtained from the rats by clipping off a small portion of the rat's tail. Since only 0.01 cc. of blood is required for the determination, it was possible to make the determinations in triplicate. The same amount of blood was taken from the controls as was taken from the experimental animals. In order to prevent further bleeding, the rat's tails were cauterized with a hot spatula immediately after the samples were taken.

As soon as one of the experimental animals died, both of its controls were sacrificed and the three animals were autopsied. Throughout the experiment, it was possible to compare each experimental animal not only with a normal rat of the same age and sex, but also with a rat which had been maintained on the same plane of nutrition.

DISCUSSION OF RESULTS

Series 85 showed very plainly that extremely low hemoglobin levels were attained in at least half of the affected rats.

Figure 1 gives the hemoglobin levels which were observed in nine affected rats, when death was about to occur. The figure also gives the hemoglobin levels in the control animals which were sacrificed at the same time. The anemias pro-

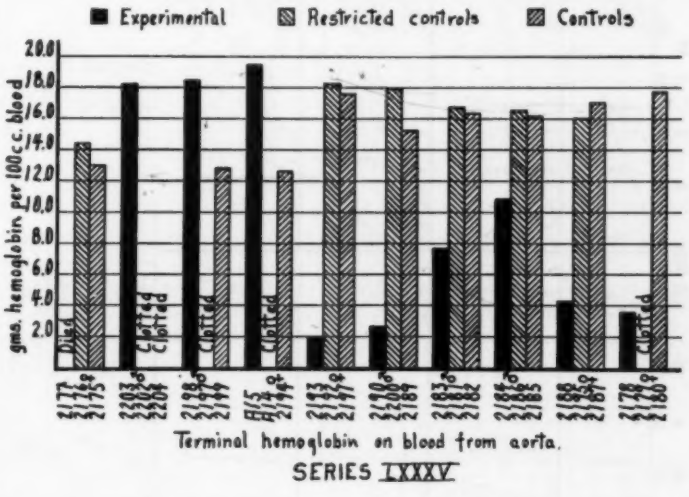

Fig. 1 Hemoglobin levels observed in moribund rats, in the order in which death occurred, series 85.

duced cannot be due to inanition because the restricted controls show no diminution in hemoglobin. The first death occurred rather unexpectedly on the eighth day of the experiment, and no blood was obtained. Of the next three rats to be sacrificed, one was killed on the tenth day and two on the sixteenth day of the experiment. Death was imminent in all three cases. Analysis showed that their hemoglobin levels were abnormally high. Whether this condition was due to the hyperactivity of the hematopoietic system or to anhydremia

remains to be determined. The last six rats (sacrificed on the thirty-fifth day to the seventy-third day of experiment) showed various degrees of anemia with hemoglobin levels ranging from 2.0 to 10.8 gm. of hemoglobin per 100 cc. of blood.

The results obtained in series 87 are shown in figures 2 and 3.

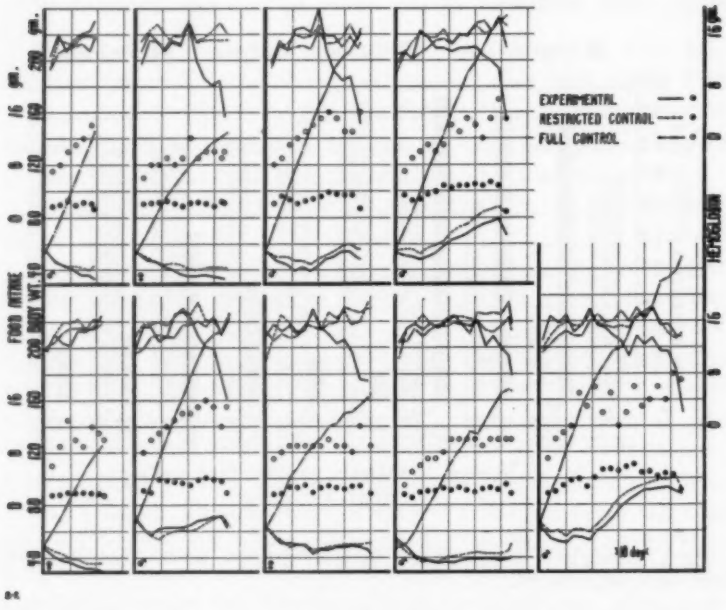

Fig. 2 Hemoglobin, growth and food intake of individual experimental rats and their controls, series 87.

Figure 2 gives the growth, food intake, and hemoglobin levels for each experimental rat together with its two controls for the entire period of the experiment. The upper curves represent hemoglobin levels, and the lower curves represent body weights, while the food intake is represented by unconnected points between the hemoglobin and weight curves in each case. The full controls had the greater food intake in

every instance. It will be noted that by the fifty-fourth day of the experiment all of the experimental rats were dead. The growth curves and food intake records are presented in order to give a clear picture of the plane of nutrition of the experimental animals and the restricted controls in comparison with the full controls. This voluntary inanition on the part of the

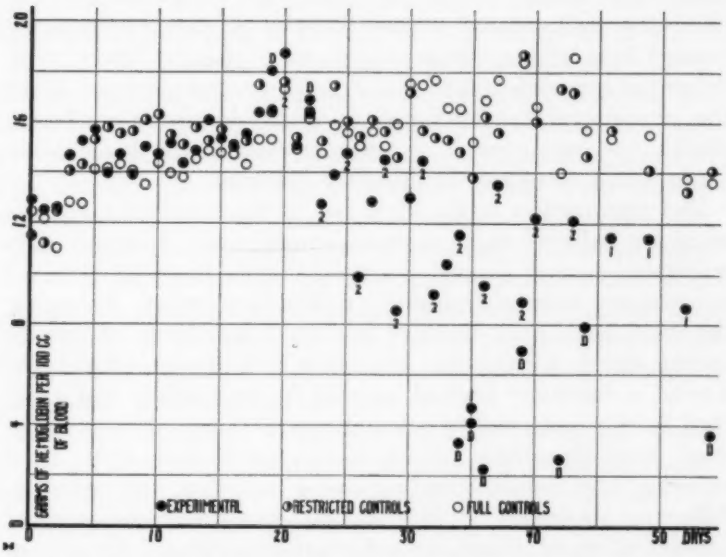

Fig. 3 Daily changes observed in the hemoglobin levels of the 27 rats in series 87. Each point represents the average for one sub-group (three rats except when otherwise indicated by sub-figures). The final hemoglobin levels reached by the nine experimentals are indicated by D.

affected rats will be discussed in another paper. It will be observed that the weights of the experimental rats and the restricted controls approximate each other.

Of the greatest interest is the record of the hemoglobin levels. In spite of the wide fluctuations which are shown in figure 2, the curves show a very definite trend.

Figure 3 presents a composite picture of the hemoglobin levels found in the rats in series 87 for the entire experi-

mental period (54 days). Each point represents the average for three rats (one sub-group as described previously) until deaths begin to occur. The points then represent 1 or 2 rats as indicated by the sub-figures. The chart shows that, beginning with the twenty-third day of the experiment, the experimental animals had hemoglobin levels which were always lower than either of the control groups. Previous to that time, the full control groups showed a definite tendency toward hemoglobin levels which were slightly lower than either the experimental or the resticted control groups. After the experimental rats began to show decreases in hemoglobin, the two groups of controls maintained hemoglobin levels which were not significantly different.

The hemoglobin levels observed in the restricted control seem to indicate that control animals will maintain substantially normal hemoglobin levels when their food intake is restricted to approximately a maintenance level. However, the work here cited involves too small a number of rats to reveal slight differences. Swanson and Smith ('32 b) observed a tendency toward anemia in rats which had their food intake restricted to a lesser degree than the rats in the work here described. Sure, Kik, and Walker ('29) have observed high hemoglobin in cases of inanition, and make the following statement: "This animal shows a typical case of concentration of blood, or anhydremia produced by a progressive marked inanition, as evidenced by the pronounced rise in the concentration of hemoglobin and erythrocytes." Sure, Kik, and Smith ('31) have also noted anemia in this connection, however, stating that "The anemias observed in three animals may be attributable to inanition, since anorexia became quite pronounced in these rats." It must be remembered that in these cases the conclusions were based upon animals which were receiving vitamin deficient diets. It is entirely possible that diverse results may also be due to the degree of inanition which is present.

The decline in hemoglobin, in series 87, occupied a large share of the experimental period. The most rapid fall took

place in 14 days and in the case of the last rat to die (54 days on experiment) the fall lasted for 34 days. It is of interest to note that in this rat, the final drop was due to an internal hemorrhage which caused the hemoglobin to fall from 8.6 to 3.6 gm. per 100 cc. of blood in 36 hours. At autopsy, the abdominal cavity was found to be filled with blood which had come from a lesion in the dorsal caudate lobe of the liver. This was the only case of its kind in either series 85 or 87.

Series 87 substantiates series 85 in that the first rats to die were not anemic. In seven out of nine rats, however, the hemoglobin levels showed a preliminary rise which was followed by a gradual decline terminated by a sudden drop to fatal levels. The pathology observed in the experimental animals was typical and has been described by Franke ('34).

There may be some objection to bleeding the rats at 3-day intervals. The fact that control animals showed no decline in hemoglobin, together with the fact that the low hemoglobin levels in series 85 were achieved without previous bleedings, would indicate that the anemias encountered are due solely to some factor in the affected wheat.

Although the blood plasma was not icteric in all cases, there was pronounced icterus in the two rats which died with high hemoglobin values. This indicated that either extensive blood destruction had begun or marked liver damage had taken place. Of the other seven rats, only two had plasmas which were not icteric at death. In these two cases, the anemia was probably due to lack of blood formation.

The high icteric indices and the jaundice found in affected rats do not necessarily imply increased blood destruction. It must be borne in mind that extensive liver damage is taking place, and that the high icteric indices, the jaundice, and the urobilinuria may be due to this factor. Jordan and Greene ('30) noted the anemias associated with both clinical and experimental jaundices and expressed the belief that the anemia was due to lack of blood formation. They stated that, although bile salts are hemolytic, their concentration in the blood stream is never sufficient to cause hemolysis. If this be

true, the anemias reported above must be due to lack of blood formation, to direct hemolytic action on the part of the toxicant, to hyperactivity of the normal blood cells destroying mechanisms, or to a combination of these factors. Experiments are being planned which will attempt to show which of the above effects is responsible for the anemia encountered.

SUMMARY

1. Death occurred in all of nineteen rats placed on an otherwise adequate diet containing toxic wheat.

2. The first six deaths occurred with no decline in hemoglobin and the last thirteen deaths occurred with hemoglobin levels ranging from 2.0 to 10.8 gm. per 100 cc. of blood.

3. The falls in hemoglobin extended over periods ranging from 14 to 34 days.

4. The anemia observed was not due to inanition.

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A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS [1,2]

IV. EFFECT OF PROTEINS ON YEAST FERMENTATION

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THREE FIGURES

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These studies on enzyme activity were started with two primary objects in mind. The first was to develop a simple biological test for certain protein toxicity described by Franke ('34), and the second to study the possible effects of these toxic proteins on several of the important enzymes of the body.

At the time these investigations were started, the only positive test available for the testing of foodstuffs for this toxicant was that of feeding trials on animals. This test gives satisfactory results, but has the disadvantage of being expensive and time consuming, besides requiring large amounts of material for a single test. To be of greatest use it is very desirable that a biological test for this toxicant possess the following qualities: 1. Economy of material; 2. rapidity of completion; 3. quantitative measurement of toxicity.

[1] Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 6 from the Department of Experiment Station Chemistry.

[2] These investigatios are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics, of the United States Department of Agriculture.

The question of the effects of the toxicant upon enzymes arose in connection with the feeding trials with rats. Early in this phase of the work, it was recognized that experimental animals ate only half as much as control animals (Franke, '34).

This voluntary food restriction certainly cannot be attributed to factors, such as monotony of diet, physical texture, lack of roughage, etc., since it took place within 24 hours after the rats were given the diet. The palatability of the diet may be questioned, but human taste and smell are incapable of differentiating between control and affected grains. There remains the possibility that the affected diet does not stimulate the appetite or digestion either by not increasing the flow of enzymes or else by rendering them inactive.

In order to get some information as to the possible effects of the toxicant on the enzymes in the animal body, it was decided to investigate the effect of the toxicant upon various enzymes in vitro.

The rate of fermentation of glucose by yeast in the presence of the material to be tested was the first enzymatic reaction to be tried, since the rate of this reaction is probably much more simple to measure than the rates of most other enzymatic reactions. It is interesting to note that Branham ('29 a, b), as well as earlier workers, made use of this enzymatic reaction to compare the antiseptic value of various compounds.

Several preliminary trials were carried out in ordinary fermentation tubes. These tubes contained sugar solution, yeast, and 1 gm. of wheat gluten. They were allowed to stand at room temperature, and at the end of 5 hours, measurements were made of the volume of CO_2 produced. The tubes containing gluten from control wheat produced 58 per cent more CO_2 than those tubes containg gluten from wheat which feeding trials had shown to be toxic.

Zeller ('26) found that solutions containing proteins caused increased rates of fermentation. He found that amino acids varied in their effects from depressing to a 100 per cent increase in rate of fermentation. Neuberg and Kobel ('26) stated that a mixture containing an amino acid and a sugar

is, as a rule, more quickly fermented by yeast than a solution containg sugar alone. They also stated that the fermentation of sugar by yeast juice is inhibited by the amino acids and urea.

Schroeder, Woodward, and Platt ('33) found that yeast poisoned by sodium iodoacetate was reactivated by addition of certain amines and amino acids, but that this reactivation seemed to be due to the change of pH when these were added, rather than a specific action of amines and amino acids.

EXPERIMENTAL

Apparatus. Although fermentation tubes, or similar apparatus, have been used by other workers for studies on the rate of fermentation, the preliminary trials on this problem were so promising that it was thought justifiable to contruct the apparatus described by Franke and Moxon ('34).

The fermentation was carried out in a thermostat at a temperature of 28°C. $\pm$ 0.05°C. Other workers have used higher temperatures for studies on the rate of fermentation. Richards ('28) found that at temperatures above 30°C. a decrease in the rate of growth of yeast cells is associated with an increase in temperature, and also that, at 30°C. abnormal, elongate cells appear which indicates that this temperature affects the bud forming process in a critical manner. On the other hand, one must not forget that division and growth of yeast cells and fermentation are altogether different processes, and that 30°C. or higher temperatures, within certain limits, may be entirely satisfactory for fermentation studies. Stier ('33) states that "Temperatures below 30° would be nearer the range of 'normal' physiological activity for yeast and similar organisms." Waksman and Davidson ('26) give the optimal temperature for zymase activity to be between 28° and 30°C.

Materials. Distilled water was used in every case where water was required. Glucose—Merck's C.P. anhydrous. Yeast—small cakes of Fleischmann's yeast were purchased from a local store twice a week. These were stored in a re-

frigerator at about 0°C. until used. Even under these precautions, variations in fermenting power of individual cakes from day to day were encountered. Therefore, controls were run with every experiment.

Proteins. Corn protein was extracted from the ground grain with 0.2 per cent NaOH according to the following method: 1000 gm. of ground grain was stirred into a battery jar containg 7 liters of 0.2 per cent NaOH and allowed to stand, with occasional stirring, for 5 hours. The supernatant liquid was then siphoned off and run through a Sharples supercentrifuge to remove all suspended particles. The protein was precipitated from the NaOH solution by adding dilute HCl until the isoelectric point was reached. The flocculated protein was allowed to settle overnight and the supernatant liquid was then siphoned off and discarded. The excess solution was removed from the precipitate by centrifuging and the protein precipitate was then dried at a low temperature. The protein was ground before being used.

Wheat gluten was prepared by soaking ground wheat in a small amount of distilled water for ½ hour and then washing the bran and starch from the gluten by kneading in several portions of distilled water. The gluten, before it was used, was dried at a low temperature and ground.

Amounts of material used. At the beginning 1 gm. of sugar was arbitrarily chosen as a convenient amount to use in this study. Likewise, 1 gm. of protein was used. In the case of yeast it was thought desirable to make a few preliminary trials before deciding on the quantity to use, as follows:

A. Control—1 gm. glucose, 1 gm. control corn protein, 20 cc. of 10 per cent yeast suspension.

B. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 20 cc. of 10 per cent yeast suspension.

C. Control—1 gm. glucose, 1 gm. control corn protein, 10 cc. of 10 per cent yeast suspension, 10 cc. distilled water.

D. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 10 cc. of 10 per cent yeast suspension, 10 cc. distilled water.

E. Control—1 gm. glucose, 1 gm. control corn protein, 5 cc. of 10 per cent yeast suspension, 15 cc. distilled water.

F. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 5 cc. 10 per cent yeast suspension, 15 cc. distilled water.

G. Control—1 gm. glucose, 1 gm. control corn protein, 2.5 cc. 10 per cent yeast suspension, 17.5 cc. of distilled water.

H. Experimental—1 gm. glucose, 1 gm. no. 523 corn protein, 2.5 cc. 10 per cent yeast suspension, 17.5 cc. of distilled water.

The yeast used was from the same cake.

The results of the above fermentation are plotted in figure 1.

The curves indicate that 2.5 cc. of 10 per cent yeast suspension gives the widest range between control and experi-

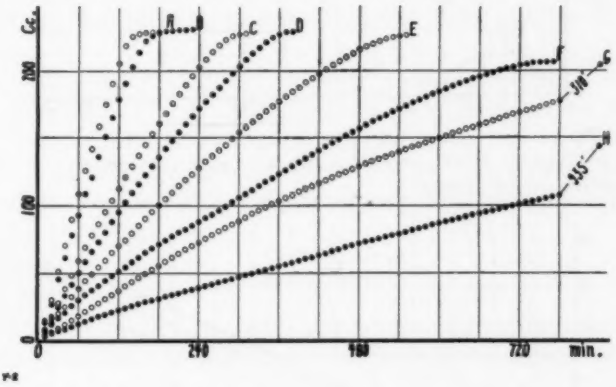

Fig. 1 The rate of CO_2 production as influenced by different corn proteins and different amounts of yeast.

mental rates of fermentation. However, the 5 cc. of yeast gives a fairly wide range and ferments much faster so that complete results can be obtained in a shorter time. Therefore, 5 cc. of 10 per cent yeast suspension was used in subsequent experiments.

Figure 2 shows the rate of CO_2 production by—

A. 1 gm. glucose, 1 gm. control corn protein, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

B. 1 gm. glucose, 1 gm. no. 523 corn protein, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

C. 1 gm. glucose, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

The curves indicate that the stimulating effect on fermentation is missing from the affected corn protein, except for the

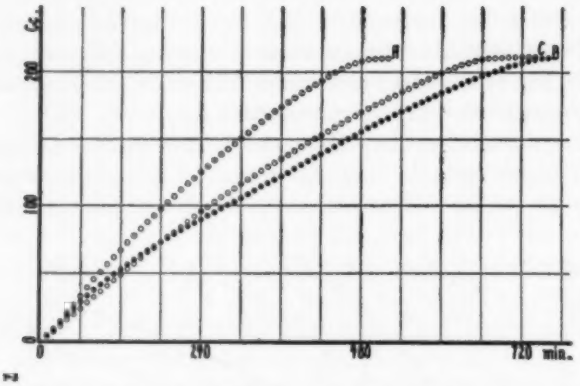

Fig. 2 The rate of CO_2 production by, A. Control corn protein. B. No. 523 corn protein. C. No protein.

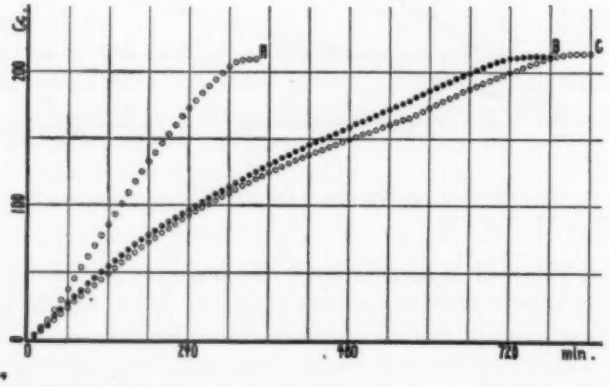

Fig. 3 The rate of CO_2 production as influenced by, A. Control wheat gluten. B. No. 582 wheat gluten. C. No protein.

first 2½ hours. This may indicate the slow inactivating of some of the yeast cells either by retardation of activity or even by death.

Figure 3 shows the rate of CO_2 production by—

A. 1 gm. glucose, 1 gm. control wheat gluten, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

B. 1 gm. glucose, 1 gm. no. 582 wheat gluten, 5 cc. 10 per cent yeast suspension and 15 cc. distilled water.

C. 1 gm. glucose, 5 cc. 10 per cent yeast suspension, and 15 cc. distilled water.

These curves show again that the protein from the affected foodstuffs does not have the stimulating effect shown by the control.

When comparison is made between control wheat and control corn protein, the wheat has the greater stimulating effect. However, one must remember that the composition of these varies.

It was early recognized, as has been observed by others, that the hydrogen ion concentration, temperature and composition of buffers when used would influence the rate of fermentation. Papers are in preparation covering these factors as well as the effect of toxic protein on other enzymes—pepsin, trypsin, invertase, etc.

SUMMARY

1. Protein from a 'normal' grain when added to a fermenting mixture of yeast and glucose will increase the rate of reaction.

2. Protein from an 'affected' grain will not increase the rate of fermentation.

3. Proteins from wheat appear to give a greater stimulating effect than proteins from corn.

4. This accelerating effect of protein varies with the protein-yeast ratio.

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THE EFFECT OF PRUNES AND THE WATER EXTRACT OF PRUNES ON THE PLASMA CO_2 COMBINING CAPACITY AND COMPOSITION OF THE URINE WHEN INCLUDED IN ACID, NEUTRAL AND UNCONTROLLED DIETS

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INTRODUCTION

The early experiments of Sherman and Sinclair ('07) and Sherman and Gettler ('12), based on analytical data, found prunes to have an excess of basic elements equal to 24.4 cc. of normal acid per 100 gm. of prunes. However, feeding experiments of Blatherwick and Long ('14 and '23) showed that prunes, though yielding a basic ash, nevertheless increased the acid formation. The decrease in urinary pH caused by eating prunes was attributed to hippuric acid resulting from benzoic acid or a precursor of benzoic acid, such as quinic, present in prunes. Brunton and Wilson ('32) stated that, contrary to expectations, both raisins and prunes, which have a high excess of alkali, produce no alkaline tide, but may produce an acid tide.

Aside from the forementioned literature, no information concerning the effects of prunes on the plasma CO_2 combining power or composition of the urine was found. In view of this absence of information correlating effects of prunes on blood plasma and composition of the urine, and in the light of the more recent findings of Fasold ('30, '31 a, '31 b, '31 c), Eimer ('31) and Bischoff et al. ('32, '34), Michalowsky ('30),

Samuel and Kugelmass ('30), and Schwartz and Dibold ('31), several experiments were conducted to determine the effects of prunes and the water extract of prunes on the plasma CO_2 combining capacity and the composition of the urine.

EXPERIMENTAL

The effect of prunes and the water extract of prunes (prune juice) on the plasma CO_2 combining power and composition of the urine was determined for a neutral, acid and uncontrolled diet. The acidities of the diets were calculated from the table of Sherman and Gettler ('12). This is not an exact method, as pointed out by Salter, Fulton and Angier ('31), and an error of 10 cc. or more of N. acid may quite commonly arise in calculating the potential acidity or alkalinity of a diet. It is also pointed out that cooking may change the acidity of a food. Eimer ('31) also indicated the danger of judging diets to be acid or basic on the basis of chemical ash analyses alone. Nevertheless, the diets used in our experiments were comparative, even though the calculations may have been in error several per cent.

An attempt was made to have a reasonably balanced diet especially in respect to edibility and nutritive value. All food was prepared for eating by the writers, except the bread.

Eight healthy young men continued on the neutral control diet for 4 days and then 6, 12, or 18 prunes or prune juice were included in the diet each day as designated in table 1. The prunes used were of the French variety from Santa Clara County of 40–50 size canned by the writers in syrup in order to obtain a uniform product. The ash of the prunes was equal to 66.32 cc. of N. base per 100 gm. of prunes. The juice used in the tests was made by extracting dried prunes with water in accordance with the procedure of Mrak and Cruess ('29). One hundred and twenty cc. of juice was equivalent to about 50 gm. of prunes and the ash was equal to 38 cc. of N. base per 100 cc. juice. An attempt was made to control the amount of water ingested and as far as possible the physical exertion of each individual. However, there

undoubtedly were variations in physical exertion that caused variations in the results obtained. We are aware of some of them. It has been pointed out by Barr ('22), Dennig ('31), Rice and Steinhaus ('31), and Bischoff et al. ('34) that exercise is a very important factor in causing variations in the alkaline reserve of the blood.

TABLE 1

Condensed table, showing the averages during the control and prune period, respectively

SUBJECTS	NUMBER OF PRUNES IN DIET	C VOLUME	C + P VOLUME	C 0.1–N TITRATABLE ACIDITY	C+P 0.1–N TITRATABLE ACIDITY	C 0.1–N ORGANIC ACID	C+P 0.1–N ORGANIC ACID	C NH_3–N	C+P NH_3–N	C pH	C+P pH	C CO_2 COMBINING CAPACITY	C+P CO_2 COMBINING CAPACITY
Acid diet													
1.–E.M.	6	1250	1574	155	242	654	785	130	142	6.11	5.81	67.44	67.08
2.–C.S.	Juice	970	1100	265	275	636	692	163	194	5.95	5.72	66.24	65.19
3.–J.F.	6	969	1358		183	606	723	203	246	5.96	5.90	63.80	64.60
4.–B.C.	12	1093	1154	238	222	556	664	133	125	5.88	5.93	59.96	61.72
5.–R.D.	18	923	1176	292	299	495	738	132	142	5.60	5.50	64.56	65.32
6.–J.D.	18	1375	1708	112	176	593	898	153	220	6.08	5.78	61.17	61.84
7.–W.C.	12	1093	1154	238	222	556	664	133	125	5.88	5.93	59.96	61.72
8.–C.H.	Juice	1370	1210	174	212	607	648	166	138	5.93	5.72	66.00	64.82
Neutral diet													
1.–E.M.	6	1369	1405	127	126	665	662	204	109	6.08	6.05	68.09	68.85
2.–C.S.	12	795	900	124	167	544	644	208	185	6.16	5.97	68.09	66.65
3.–J.F.	18	915	1246	151	124	507	714	102	143	6.04	5.99	64.96	65.66
4.–B.C.	Juice	1210	1090	169	135	630	590	149	59	5.95	5.82	61.78	62.73
5.–R.D.	18	1065	1000	156	218	521	545	100	104	5.98	5.74	63.23	63.88
6.–J.D.	12	1197	1470	108	128	541	617	172	177	6.08	5.91	67.61	66.47
7.–A.L.	6	1500	1457	107	102	660	620	176	144	6.14	5.77	63.10	68.07
8.–C.H.	Juice	829	1140	126	142	540	590	206	95	5.70	5.90	64.40	66.60

C = on control diet.

C + P = on control diet plus prunes.

Urine samples were collected for the 24-hour period starting after breakfast and ending before breakfast. Blood samples were drawn between 5.00 and 8.00 P.M. except for subjects 5, 6 and 7 on the last day. Blood samples were taken from three individuals on the last day of the neutral diet to determine the changes occurring in plasma CO_2 combining capacity at various intervals after the ingestion of prunes.

The procedure used for the experiment on the acid diet was similar to that used for the neutral diet except that extra blood samples were not taken the last day. A glass of water was included between meals and after dinner.

The calculated excess acidity for the diet was more than 50 cc. N. acid. This is a less acid diet than employed by Bischoff et al. ('34), but more acid than used by Fellers et al. ('33) which we calculated to be equal to 11.2 cc. N. acid. The diet used by Chaney and Blunt ('25), Saywell ('32 a, b and '33), and Saywell and Lane ('33) is equal to about 20.42 cc. of N. acid according to our calculations from the tables of Sherman and Gettler.

In the uncontrolled diet no attempt was made to control the activities of the men or the food they ate. A record was kept of the food eaten at each meal and a rough estimation was made of the acid or base of the meal based on Sherman and Gettler's tables. The averages of these results are included in table 2 under the designations of A for acidic, B for basic and N for neutral. The letters A or B alone indicate

TABLE 2

Condensed table, showing the averages for the uncontrolled diet

SUBJECTS	NUMBER OF PERIODS IN DIET	ACID BASE OF DIET		C VOLUME	C + P VOLUME	C 0.1–N TITRATABLE ACIDITY	C+P 0.1–N TITRATABLE ACIDITY	C 0.1–N ORGANIC ACID	C+P 0.1–N ORGANIC ACID	C NH_3–N	C+P NH_3–N	C pH	C+P pH	C CO_2 COMBINING CAPACITY	C+P CO_2 COMBINING CAPACITY
		C	C+P												
1	18	A ±	A ±	1003	935	13	21	640	534	20	37	6.48	6.77	66.66	66.00
2	Control	N	N	1217	1278	84	127	572	492	43	68	6.13	6.37	64.42	63.60
3	12	A +	A +	1854	2020	110	176	528	550	29	52	5.75	6.14	65.45	65.74
4	12	A +	A	587	492	107	135	395	347	11	41	5.89	5.84	61.50	59.76
5	18	A ±	A +	1420	1517	217	229	575	589	45	113	5.72	5.69	64.10	62.66
6	6	A +	A +	2148	2087	171	133	808	754	66	123	6.09	6.08	63.70	59.73
7	Juice	A +	A +	1970	2100	162	208	626	735	123	79	6.16	6.11	62.92	62.76
8	Juice	A ±	A +	993	1245	207	79	431	569	40	53	5.56	6.20	64.45	64.54
9	Juice	A −	A −	1153	1515	111	184	734	663	24	59	6.25	6.07	67.17	63.72
10	6	A	A ±	932	972	66	−14	409	558	24	56	6.13	6.69	62.49	59.19
11	Control	A ±	A +	1013	1034	140	102	479	520	43	57	6.26	6.51	67.93	66.12
12	Control	A +	A ±	1647	1995	141	160	760	777	57	191	6.17	6.34	68.49	64.10

Subject 8 fasted 3 days during the control period.
Subject 1 found to be a $NaHCO_3$ addict.
Subject 10 took $NaHCO_3$ on two days.

a very mildly acid or basic meal, whereas the presence of one + indicates acidic or basic food equal to about 10–25 cc. of N. acid or base. Two + marks (i.e., A ++) indicates an acid or basic meal stronger than 25 cc. of N. acid or base. N — indicates an acid to neutral meal and N + indicates a neutral to basic meal. Most of the meals ingested during the period of this diet were acid according to our calculations. Twelve men ranging in ages from 18 to 72, engaged in working in a prison hospital, were included in the diet for a period of 15 days. The first 7 days was a prune free period to obtain an idea of the normal variation in the plasma CO_2 binding power and composition of the urine. During the second period of 8 days various amounts of prunes or 120 cc. of prune juice was included in the diet of nine of the subjects. Three of the men continued through the second period without including prunes or prune juice in their diet. Urine samples were collected for the 24-hour day starting after breakfast and ending before breakfast the next morning. Blood samples were taken at 9.00 A.M. each morning.

Analytical determinations were all made shortly after the collection of the samples. All determinations were made in duplicate or triplicate, except those of the ammonia, which in most cases were single determinations. The following methods were employed: pH by the quinhydrone electrode; organic acids, Van Slyke and Palmer ('20); ammonia, Van Slyke and Cullen ('14); titratable acidity, Henderson ('11); and plasma CO_2, Van Slyke and Cullen ('17), as described by Hawk and Bergeim ('31), page 489.

RESULTS AND DISCUSSION

Neutral diet. The results obtained for the neutral diet are given in a summary of the averages in table 1.

There was considerable variation in volume excreted even though an attempt was made to control it by regulating the daily intake of water and the activities of the men. These variations undoubtedly had some effect on the pH values of the urine by changing the buffer relations; the reasons for

this, however, are beyond the scope of these investigations. The average pH values for the control periods had an extreme variation of 0.46 pH units which is 0.05 less than the extreme variation for any individual during that period. In most instances the extreme individual variations for the control period were not over 0.27 pH units. The pH values for the control periods all fall within a range of 5.7 to 6.16. This appears to be considerable variation; however, the urinary pH values of the individual subjects varied, some being consistently lower and others higher. The writers believe a fairly stable state was reached when prunes were included in the diet. In all instances when prunes were included in the diet there was a drop in the pH of the urines. In no instance, however, did the average pH of the urine of an individual for the control and prune (C + P) period, fall below the lowest average pH obtained for an individual (subject 8) during the control period. The changes obtained when juice was included in the neutral diet were not consistent. Subject 8 showed an average increase of 0.2 pH units and subject 4 an average decrease of 0.13 pH units. These changes are probably not significant. There was no direct correlation between the pH of the urine and the amount of prunes ingested on the neutral diet. It is possible that if more men had been used for each particular test the variations would have been more consistent; however, since the variations were so wide we do not believe it would have made any difference.

The titratable acidity of the urine did not change consistently. Variations were observed, but are believed to have been within the normal ranges. More significant is the organic acid output. A marked change in organic acid excreted was observed when twelve and eighteen prunes were included in the diet in all instances except in the case of subject 5. Only slight changes in organic acid excreted occurred when six prunes or 120 cc. of prune juice were included in the diet. It should be pointed out here that, according to the recent work of Fasold ('30), there is a relation between the organic

acid excreted and the kind of food eaten. He indicated that organic acids, as citric and lactic, are mostly oxidized and that a relation exists between the amount of basic constituents of foods ingested and organic acid excreted. The increased organic acid excretion observed when twelve and eighteen prunes were included in the neutral diet was not caused by the presence of benzoic acid, according to findings of Blatherwick and Long ('23). They believed the increase in organic acid excretion occurring upon the ingestion of prunes to be caused largely by quinic acid in the prunes. Fellers et al. ('33) attributed the increase in organic acid excretion caused by cranberries largely to quinic acid in the fruit. Quick ('31) found quinic acid to be a precursor of benzoic acid which is excreted as hippuric acid. In the absence of more definite information regarding the quinic acid of prunes, it appears more reasonable to interpret the increase in organic acid excretion caused by eating prunes in the light of the findings of Fasold ('30, '31 a '31 b, '31 c). According to his interpretations, the increased excretion of organic acids observed when twelve and eighteen prunes were ingested was caused by the excess alkalinity of the prune plus neutral diet even though some hippuric acid forming substances as benzoic acid were present in the prunes. As previously stated, prune ash has a relatively high alkaline value, whereas the amount of benzoic acid, as given by Radin ('14), and the small amount of quinic acid probably present hardly seem sufficient to account for the marked increase in hippuric acid excretion observed. Further studies on hippuric acid and urinary pH changes are being made and will be reported at a later date.

In regard to the NH_3 eliminated during the neutral diet, the most marked decrease was observed in the case of those ingesting prune juice. However, the subjects ingesting prunes showed a slight decrease, if any, in the free ammonia. In the light of Fasold's findings, there must have been sufficient alkali in the prunes and the prune juice to replace the difference in the NH_3 excreted when the NH_3 excretion decreased after including prunes in the diet. Fasold and others have

shown that NH_3 is called upon for the neutralization of acids to some extent and acids are called upon for the neutralization of bases to some extent. This agrees with the forementioned interpretation of the observations on organic acids excreted.

At this point it may be stated that the quantities of prunes fed had no significant effect on the acid-base equilibrium. However, because of the conclusions of Bischoff et al. ('34), Fasold ('30) and Shock and Hastings ('34), such a statement might be questioned. The blood CO_2 combining power of the blood plasma was determined. In no case did the inclusion of prunes cause a variation greater than that which has been observed in healthy normal individuals; Shock and Hastings ('34), Cullen and Earle ('29), Cullen and Robinson ('23), Dodds and McIntosh ('23), and Meyers and Decker ('24). The greatest variation of the average CO_2 combining capacity during the control and control plus prune diet periods was plus 5 volumes per cent. The larger part of this variation may be attributed to the limit of accuracy of the technic used. In order to determine the variations in the plasma CO_2 combining capacity at various periods during the day, three men remained on the neutral diet an extra day. Blood samples were drawn at 9.00 A.M. after breakfast, but before the ingestion of prunes, and at 10.00 A.M., 11.00 A.M. and 2.30 P.M. Six, twelve or eighteen prunes were ingested by each individual soon after the blood sample was taken at 9.00 A.M. The variations observed were in accord with the day to day variations of the plasma CO_2 combining capacity. Even when eighteen prunes were ingested, no significant change in plasma CO_2 combining capacity occurred. From these results it may be concluded that eighteen prunes (200 gm.) or 120 cc. prune juice have no significant effect on the plasma CO_2 combining power, in healthy individuals, when included in a neutral diet. Other factors undoubtedly play an important part in maintaining the acid-base equilibrium, but it is beyond the scope of this study to go into these, though the writers are aware that some of these factors may have had some effects on the results obtained.

Acid diet. The same subjects that were on the neutral diet were placed on an acid diet, with the exception of subject 7. The data obtained are also given in table 1.

The results obtained were very similar to those obtained on the neutral diet. The urinary pH changes were about the same as those on the neutral diet. The titratable acidity on the acid diet was higher than that of the neutral diet, the organic acids were slightly higher on the acid diet than on the neutral diet. The NH_3 excretion was somewhat less on the acid diet than on the neutral diet. In regard to the CO_2 combining power of the blood plasma, the results obtained were very similar to those of the neutral diet. The inclusion of prunes in the acid diet did not cause a significant lowering in the plasma CO_2 combining capacity. It is rather interesting to note that the averages of the CO_2 combining capacity for each individual during the acid control period and the acid control plus prune period varied within the same range of volume per cent as the same periods of the neutral diet. It may be concluded that eighteen prunes or 4 ounces (120 cc.) of prune juice per day do not produce any significant change in the plasma CO_2 combining power when included in an acid diet equal in acidity to the one used.

Uncontrolled diet. Discussion thus far has been restricted to the controlled diets. While controlled diets alone may be sufficient in this type of investigation, it was considered interesting and desirable to supplement these with an uncontrolled diet. To our knowledge, such supplementary work has not been reported. Twelve men of various ages and eating habits were placed on an uncontrolled diet. The ages of the men were as follows: Subject 1, 63 years; 2, 61 years; 3, 57 years; 4, 39 years; 5, 42 years; 6, 23 years; 7, 27 years; 8, 72 years; 9, 23 years; 10, 18 years; 11, 49 years; 12, 23 years. These men were known to have no metabolic disorders. The study of urinary composition and blood alkaline reserve and the effect of prunes on them in such a group of individuals presents interesting data.

The data obtained are given in table 2. Before considering the general data in table 2, a few interesting observations should be mentioned. Subject 10 was found to have taken at least one $NaHCO_3$ tablet in the forenoon of the ninth and eleventh days of the diet. On these days the pH values and organic acids of the urine increased and the total acid of the urine decreased. These changes are in accordance with Fasold's findings. The changes in NH_3 excretion, however, were not significant. Even more interesting was the fact that the plasma CO_2 combining power did not vary any more than when $NaHCO_3$ was not ingested. Subject 1 was found to take $NaHCO_3$ every day. The pH values and organic acids of the urine were consistently high and the total acids excreted were consistently low. The results obtained for NH_3 excreted were also indicative of his habit since they were consistently low. The plasma CO_2 combining power, however, was but slightly higher than for most other subjects. The averages were lower than several others.

Subject 8 fasted during the fourth, fifth and sixth days. There was a sharp drop in the pH of the urine reaching 5.3 on the third day of fasting. The decrease in plasma CO_2 combining power was about 10 volumes per cent on the second day. This is to some extent in accordance with the work of Walinski ('26), who found that a 24-hour starvation reduced the alkaline reserve of human blood about 7 per cent and 18 per cent after 48 hours of starvation.

The average diet of these twelve subjects was slightly on the acid side in most instances. The titratable acidity, organic acids and NH_3 of the urine varied within normal limits. The pH values in all cases except those already discussed were within a range similar to that obtained on the controlled diets. The inclusion of prunes did not consistently change these results in one direction or the other. The plasma CO_2 combining power changed very little in one direction or the other and these changes were within the range of variations of the men who remained 14 days on the prune free diet. In the diet free of prunes the maximum variation in plasma CO_2

combining power was from 57.66 to 72.2, or 14.54 volumes per cent. The greatest urinary pH variation was 5.7 to 7.1, or 1.4 pH units. The maximum variation in plasma CO_2 combining capacity for an individual including prunes or prune juice was 53.39 to 69.26, or 15.87 volumes per cent for subject 9, who included prune juice in his diet. The greatest urinary pH variation was 1.87 for the same individual. These figures are given merely to indicate the magnitude of the variations and not to correlate with the ingestion of prunes or prune juice. The extreme variations given for subject 9 all occurred in the diet plus prune juice period.

SUMMARY AND CONCLUSIONS

1. The effects of six, twelve and eighteen prunes and 120 cc. (4 ounces) of prune juice on urinary composition and plasma CO_2 combining power when included in neutral, acid and uncontrolled diets were determined. Two men on the uncontrolled diet ingested $NaHCO_3$ and one man fasted for 3 days during the diet and the effects on the urine and plasma CO_2 combining power were noted.

2. The inclusion of twelve and eighteen prunes in the neutral and acid diets caused an increase in organic acids and a decrease in NH_3 and total acids excreted in most instances. When prunes were included in the diet the urinary pH values dropped in most instances. According to the interpretation of Fasold, the changes in organic acids, total acids, and ammonia were caused by the alkalinity of the prunes, the non-oxidizable benzoic and quinic acids being responsible for a small proportion. The changes occurring when prunes were included in the uncontrolled diet were not significantly different from the changes occurring when prunes were omitted from the uncontrolled diet.

3. The plasma CO_2 combining power was not changed significantly when six, twelve or eighteen prunes or 120 cc. of prune juice were included in the neutral, acid or uncontrolled diets. Variations in plasma CO_2 combining power at various periods during the day before and after the inclusion of prunes in the neutral diet were slight.

4. The variations in plasma CO_2 combining power were within the normal range as established by various authorities. The greatest variation in plasma CO_2 combining power and urinary pH occurred on the uncontrolled diet. These variations were not caused by prunes.

5. The changes in urinary composition caused by the ingestion of $NaHCO_3$ were in accordance with the findings of Fasold. Fasting caused a drop in urinary pH and plasma CO_2 combining power.

6. The writers are aware that certain factors as R.Q. and and renal function were not taken into consideration; however, they believe since great care was taken picking subjects and conducting the experiments they were sufficiently controlled and show that the inclusion of as much as eighteen prunes (200 gm.) in the diet does not cause a significant variation in one direction or the other in the plasma CO_2 combining power, but on the uncontrolled diet tend to narrow the range of variation. They also offer an explanation for the changes occurring in urinary composition when this fruit which has a highly alkaline ash is eaten.

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HUMAN MILK STUDIES

XVI. VITAMIN D POTENCY AS INFLUENCED BY SUPPLEMENTING THE DIET OF THE MOTHER DURING PREGNANCY AND LACTATION WITH COW'S MILK FORTIFIED WITH A CONCENTRATE OF COD LIVER OIL [1] (A TEST ON RACHITIC INFANTS AND RATS)

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FOUR PLATES

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Clinical experience demonstrates that, under present living conditions, neither human milk nor cow's milk can always be relied upon to protect babies against rickets, although rickets is universally less frequent among nursing infants than among bottle-fed babies. Despite the fact that specific treatment for the prevention and cure of this disease has been known for several years and many antirachitic agents are not only in the hands of the physicians and public health workers, but are being distributed widely to the layman, recent observations have shown that 26 per cent of breast-fed and 56 per cent of the formula-fed babies living in our city have rickets (Barnes, Brady and James, '30).

Laboratory biological tests have shown that ordinary breast milk is a poor source of vitamin D, since a supplement of 20 cc. of woman's milk daily did not protect a rat of usual age and weight from rickets (Hess and Weinstock, '27); even 25 to

[1] The National Oil Products Company, Harrison, New Jersey was kind enough to furnish the fortified cow's milk for the mother and to pay for the breast milk used in this study.

40 cc. of pooled human milk of women on the average American dietary failed to produce healing in rachitic rats (Outhouse, Macy and Brekke, '28). This fact that mother's milk contains very little of the anti-rachitic factor when given to the rat as the universal experimental test animal for rickets becomes of greater importance when it is borne in mind that there is an essential difference between the reaction of the infant and the rat in regard to response to unitage of vitamin D (Barnes, Brady and James, '30).

The biological potency of breast milk can be enhanced up to a specific physiological limit by the addition of a concentrated source of the vitamin B complex (Donelson and Macy, '34) and vitamin A (McCosh et al., '34) to the average dietary and that mother's milk produced during an inadequate dietary regime may fall short of meeting the optimal concentration of vitamin A in milk, and, therefore, the physiological needs of the infant (Macy, et al., '28; Thatcher and Sure, '32; Kennedy, et al., '23). It does not seem improbable that woman's milk may be fortified in respect to vitamin D by the administration of an antirachitic substance under propitious dietary conditions. It is desirable to have as complete an understanding as possible, based on investigations in the clinic as well as in the laboratory, of the relationship of woman's milk, produced under dietary supplements of the current antirachitic substances, to the development and cure of rickets.

There is a tendency at present to exaggerate the protective value of breast milk against rickets and dental disturbances when the mother takes in her daily diet any one of a number of vitamin D preparations. Clinical proof does not exist for some of the present day advertising in this regard. This may be harmful to the child, as it has led mothers to believe that it is unnecessary for the nursing infant to take directly supplementary additions of vitamin D.

There is apparently a variation in the degree of individual response of women to specific medication as well as to different types of antirachitic treatments for it is shown that babies do not enter the world with an equal susceptibility to rickets

(Hess, '29). Furthermore, in clinical tests woman's milk can be endowed adequately with vitamin D by exposing the nursing woman to ultra-violet light as demonstrated by the healing of rickets in infants, whereas a similar enhancement of human milk cannot be brought about by giving to nursing mothers one tablespoonful of cod liver oil in addition to a satisfactory diet (Gerstenberger et al., '27).

The present report is a part of a series of studies on the effectiveness of diets rich in vitamins on the nutrition of the woman during pregnancy and lactation, on the biological potency of the milk secreted and on the extent to which the vitamins are transmitted to the young through the placenta and through the milk.

EXPERIMENTAL

In view of the fact that women respond differently to various types of antirachitic treatment, in this study of the vitamin D potency of mother's milk as influenced by supplementing the diet during pregnancy and lactation with cow's milk fortified with a concentrate of cod liver oil, a woman [2], who had demonstrated herself previously to have a capacity for a large milk flow, was chosen as the source of supply for the milk used in this investigation. This assured an experimental milk of a constant source and quality for both the control and experimental babies. Her diet over a period of detailed metabolic studies throughout two former reproductive cycles was adequate according to accepted dietary standards. In addition to the well-selected diet, this mother chose to take continuously for this study from the nineteenth week of pregnancy and throughout lactation 2 quarts of milk fortified with a cod liver oil concentrate containing a total of 300 units of vitamin D per day.[3]

[2] A continuous acid-base mineral balance on this mother from the nineteenth week of pregnancy through eight weeks of lactation will be reported in the near future.

[3] The vitamin D milk furnished the mother was assayed by C. A. Hoppert, Ph.D., Department of Chemistry, Michigan State College.

The mother's own infant served as the control and the three experimental babies were selected from breast-fed infants who had developed severe clinical rickets as shown by roentgen examinations and by determinations of the blood calcium and phosphorus. The control baby had monthly x-rays from birth through the 8th month, whereas the experimental rachitic babies had roentgenograms and bloods taken immediately preceding the introduction of the experimental milk and at frequent intervals thereafter.

RESULTS AND DISCUSSION

Table 1 and plate 1 demonstrate that a mother who continuously consumed from the nineteenth week of pregnancy through 8 months of lactation a generous mixed dietary enriched daily with 2 quarts of milk to which 300 units of vitamin D in the form of a concentrate of cod liver oil were added, was able to bear and rear an infant (J. R.) who showed no clinical signs of rickets during the first 8 months of life, including the rachitic season. Adequate protection was given to the infant, therefore, either by a transfer of vitamin D through the placenta or the milk, or both. In addition, and perhaps of even greater significance, a more favorable provision for bone growth was furnished by a superior maternal mineral metabolism. According to the results obtained during a continuous mineral metabolic balance of the mother during her present reproductive cycle, she was retaining more than twice the average amount (Macy and Hunscher, '34) of bone building constituents. [4]

On the contrary, three colored breast-fed babies who had severe clinical rickets as shown by roentgen examinations and by chemical determinations of blood calcium and phosphorus showed no improvement when placed for periods varying from 11 to 43 days on 30 to 32 ounces daily of breast milk produced by the mother in this study (table 1 and plates 2 to 4). Insufficient amounts of vitamin D were transmitted through the breast milk alone to induce healing of rickets in these three

[4] See footnote 2, page 649.

babies although the same milk simultaneously prevented the occurrence of rickets in the mother's own baby. The vitamin D potency and the ineffectiveness of this particular breast milk that was produced under superior dietary conditions was further verified by appropriate curative tests on rachitic rats in which it was shown to contain little more than a trace of vitamin D.[5]

TABLE 1

Blood and x-ray studies on rachitic infants receiving breast milk from a mother whose diet was supplemented with cow's milk fortified with 300 units of cod liver oil

SUBJECT	AGE AT BEGINNING	DATE	WEIGHT	NUMBER OF DAYS ON NEW BREAST MILK	TOTAL BREAST MILK TAKEN	CALCIUM	PHOSPHORUS	Ca X P PRODUCT	X-RAY DIAGNOSIS OF RICKETS
Control	*Wk.*		*Kg.*		*Oz.*	*Mg./100 cc.*	*Mg./100 cc.*		
J. R. (white)	19	11/ 9/33	6.98	....	....	....	....	....	Normal
	35	3/ 5/34	9.00	....	3920	....	....	....	Normal
L. C. (colored)	53	11/17/33	8.82	0	....	9.82	2.32	22.8	+++
		11/21/33	....	4	....	9.63	2.21	21.3	....
		11/28/33	....	11	....	10.09	2.64	26.6	....
		12/12/33[1]	9.36	23	832	9.26	2.66	24.6	+++
V. W. (colored)	21	1/12/34	6.44	0	....	9.82	2.82	27.7	++++
			....	12	....	10.20	2.90	30.0	....
		2/16/34	7.29	24	928	8.27	3.63	30.0	++++
J. O. (colored)	36	1/24/34	9.00	0	....	8.43	3.57	30.0	+++
		2/ 6/34	9.08	13	....	10.26	....	....	....
		3/13/34	8.90	43	1296	10.50	3.47	36.4	++++

[1] After 1 month healing was evident and complete in 2 months on evaporated milk to which 150 units of cod liver oil concentrate had been added.

When the same concentrate of vitamin D was given directly to the infants in a cow's milk formula, healing of rickets was evident in less than a month and was complete in 2 months

[5] The biological tests on the breast milk were kindly conducted by Henry C. Sherman, Ph.D., and Theodore F. Zucker, Ph.D., both of Columbia University, New York City.

(plates 2 to 4). Exclusive of the advantage to the mother and her own infant, direct administration of vitamin D to the rachitic infant is more effective than the indirect method via breast milk. Of preeminence is the fact that the infant, whose mother's diet was known to be superior not only in vitamin D, but in minerals and proteins of excellent quality during both pregnancy and lactation, was able to build normal bone up to the eighth month of life with no direct administration of vitamin D.

SUMMARY

A woman whose diet was superior in quality and, in addition, was fortified with 2 quarts of cow's milk daily to which 300 units of a vitamin D concentrate of cod liver oil were incorporated, was unable to secrete a breast milk that was sufficiently enriched with vitamin D to heal rickets in three colored breast-fed infants or in experimental rachitic rats. Her own breast-fed baby, however, showed no signs of rickets throughout the investigation.

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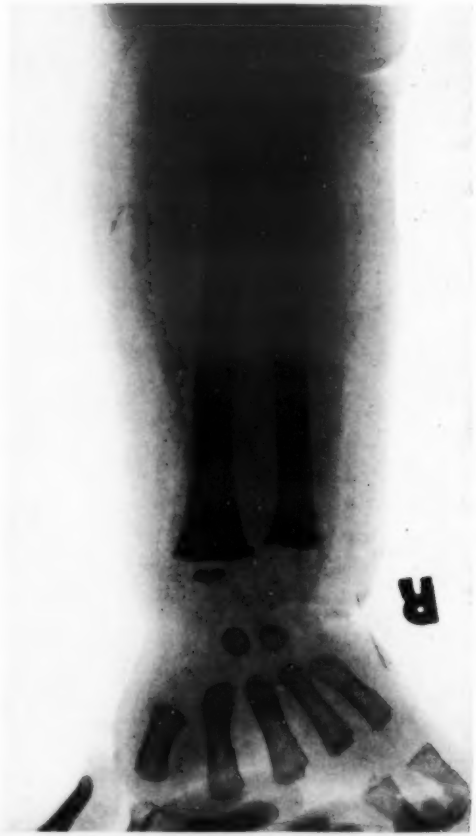
J. R.

2/23/33



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DONALD J. BARNES AND OTHERS

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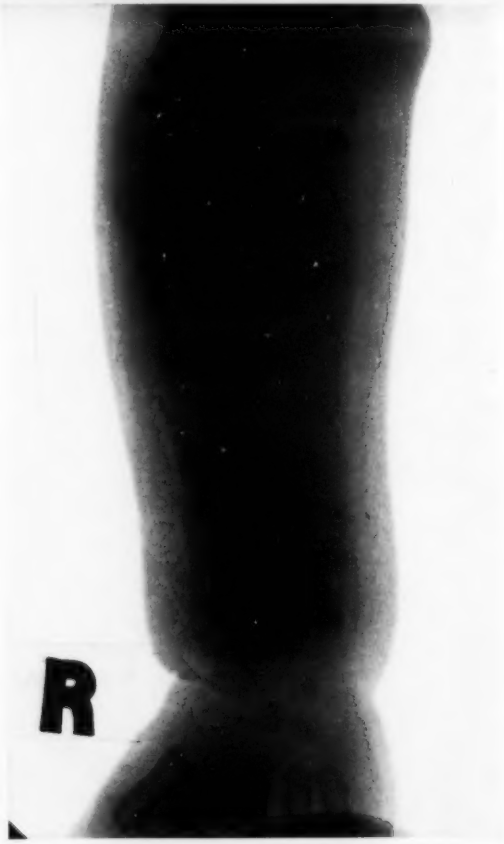

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INORGANIC SALTS IN NUTRITION

IX. CORRELATION BETWEEN SUPPRESSED GROWTH AND THE DEVELOPMENT OF POLYCYTHEMIA INDUCED BY FEEDING A RATION POOR IN SALTS [1]

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TWO CHARTS

(Received for publication April 23, 1934)

It has recently been demonstrated (Swanson and Smith, '32 a, b) that it is possible to induce a severe and true experimental polycythemia in the rat by greatly reducing the inorganic residue of an otherwise adequate ration when casein serves as the dietary protein. Upon such a regimen the number of red cells in the blood of the experimental animals increased some 60 per cent in 3 months, whereas the increment observed in the blood of the normal rats receiving a sufficient quantity of a favorable salt mixture in the diet was only 27 per cent. Furthermore, the erythrocytes were subnormal in size and, in contrast to the usual picture in clinical poly-

[1] Some of the data reported in this paper are taken from a dissertation presented by Pearl P. Swanson in partial fulfillment of the requirements for the degree of doctor of philosophy, Yale University, 1930. Other parts represent findings in the extension of the study at Iowa State College. Grateful acknowledgment is made for the technical assistance rendered by Ernestine Frazier and Gladys Timson, Iowa State College.

The expenses of this research were defrayed in part by a grant from the Russell H. Chittenden Fund for Research in Physiological Chemistry, Yale University.

The preceding article of the series may be found in the J. Biol. Chem., vol. 105 p. 181.

[2] Alexander Brown Coxe Fellow in Physiological Chemistry, Yale University, 1929–1930.

cythemia, the concentration of hemoglobin in the blood was subnormal.

In the early experiments, the polycythemia occurred in practically every animal of a fairly large group receiving the defective ration. Later, however, when the investigation was extended, it was found in many instances that only a mild polycythemia had been induced and that in certain cases, the blood picture was entirely normal. The present report deals with certain correlations bearing on these observations.

SUPPRESSION OF GROWTH AND THE INCIDENCE OF POLYCYTHEMIA

Rats of an average body weight of 160 gm. were employed in the studies in order to obtain sufficient quantities of blood for analytical purposes. Therefore, due to the stunting effects of the diet, a preliminary period of growth upon an adequate ration was necessary before it was possible to inaugurate the low salt feeding (Swanson and Smith, '32a). Accordingly, the experimental ration was not offered until the rats had attained a body weight of 120 gm. After maintenance upon this diet for 3 months, the adult rats were as definitely polycythemic as were very young animals after 1 month (Smith and Schultz, '30). It was noted, however, that with the larger rats the administration of the defective ration did not produce the immediate suspension of growth that occurred when the low salt diet was given to the newly-weaned animals (Winters, Smith, and Mendel, '27). An analysis of the growth performance of the rats used in the present study showed that rats varied in their response to the deficient diet. Although all weighed 120 gm. at the initiation of the experiment, some individuals attained a final body weight of about 160 gm.; others, 180 gm.; and still others, 200 gm. The entire group of experimental animals (sixty-five rats), classified according to their body weight, fell into one or the other of the three weight groups indicated above. The rats in group I (158 $\pm$ 12.4 gm. [3]) grew at a retarded rate for a period of 32 days, attaining an average weight of 158 gm.

[3] Standard deviation.

No further increments in growth occurred thereafter and the body weight was maintained at this level for the remainder of the experimental period. The individuals of group II also increased in weight, but the subnormal growth extended over a longer interval than in the case of the animals of group I (56 days vs. 32 days). Growth then ceased and the body weight (183 ± 8.8 gm.) remained constant for the next 32 days, after which the experiment was terminated. The growth of the animals in group III was never completely inhibited. They grew slowly for nearly the entire experimental period. During the last 2 weeks they showed a tendency to lose weight. However, the average body weight at the end of the period was still high, i.e., 199 ± 7.8 gm. A biometric interpretation [4] of the growth of the three groups of rats receiving the ash-poor ration is recorded in chart 1.

Inasmuch as the polycythemia described by Smith and Schultz ('30) occurred after a period of maintenance of constant weight upon the low salt diet, it seemed likely that the varied growth performance of our rats might be related to the different degrees of severity of polycythemia observed. It was found that rats maintaining an average body weight of approximately 158 gm. for 58 days (group I) had an average of twelve million red cells per cubic millimeter of blood; those holding a body weight of 180 gm. for 32 days (group II), ten and seven-tenths million; and those that grew for the entire experimental period (group III), nine and nine-tenths million. Thus, it is evident that the degree of polycythemia is correlated with the suppression of growth of the test animal fed the salt-poor ration. Whenever growth occurs at a constant, though minimal, rate, the blood picture remains normal in spite of the imposed dietary deficiency (group III, chart 1). In order to induce an increment in the number of red cells during a 3-month period that is twice the normal, an inhibition of growth must occur early in the experimental history (after

[4] The solid middle lines show the mean growth curves of the groups. The standard deviations from the average weight of the respective intervals are represented on each curve by the dotted line on each side of the average.

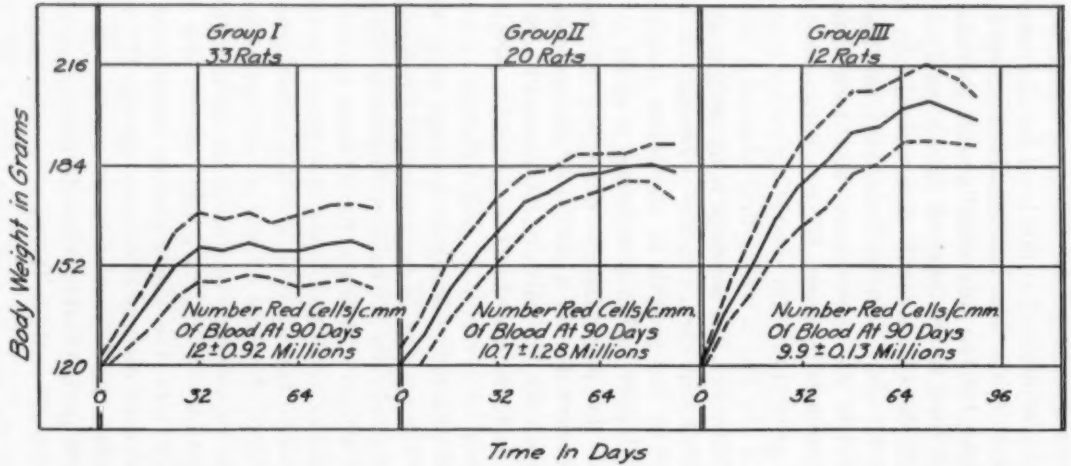

Chart 1 Variation in the growth response and the erythrocyte count of a group of sixty-five rats placed upon a low salt ration upon the attainment of a body weight of 120 gm.

1 month) with maintenance of weight thereafter (group I, chart 1).

The importance of a protracted period of maintenance of constant weight for the production of the polycythemia is illustrated in the results obtained in the continuation of the studies at Iowa State College when a different strain of animals was used (Wistar, strain B). A comparison of curves A and B in figure I, chart 2, shows that although the Iowa rats stunted near the desired level of 160 gm., constant weight (defined as $x \pm 10$ gm.) was maintained for a period only approximately one-half as long as was the case in the Yale rats. The average erythrocyte concentration in the blood of these rats was ten and seven-tenths million per cubic millimeter, a value only slightly above normal (nine and seven-tenths million). Three rats were chosen from this group and maintained upon the defective diet for another month. The red cell count rose from values of ten and three-tenths, ten and five-tenths and nine and eight-tenths million per cubic millimeter of blood at the end of 3 months to twelve and one-tenth, eleven and nine-tenths, and ten and nine-tenths million, respectively, when the experimental ration was given for this additional time. A representative case is depicted in figure II, chart 2. This experiment suggests that if a rat responds to the low salt diet in such manner that the body weight is not held at a constant level for at least 7 to 8 weeks, the experimental period must be lengthend. The practical drawback of this situation from the point of view of experimentation is at once apparent.

However, with a slight modification of experimental technic, it was possible to obtain the same average type of curve in the second laboratory that was typical of the growth of the rats grown at Yale, without lengthening the time of the experiment. Furthemore, the polycythemia was just as severe. The effects induced by the feeding of the low salt food were duplicated despite the fact that the animals employed were taken from a colony of a new strain maintained upon a different stock ration. Instead of keeping the animal upon the

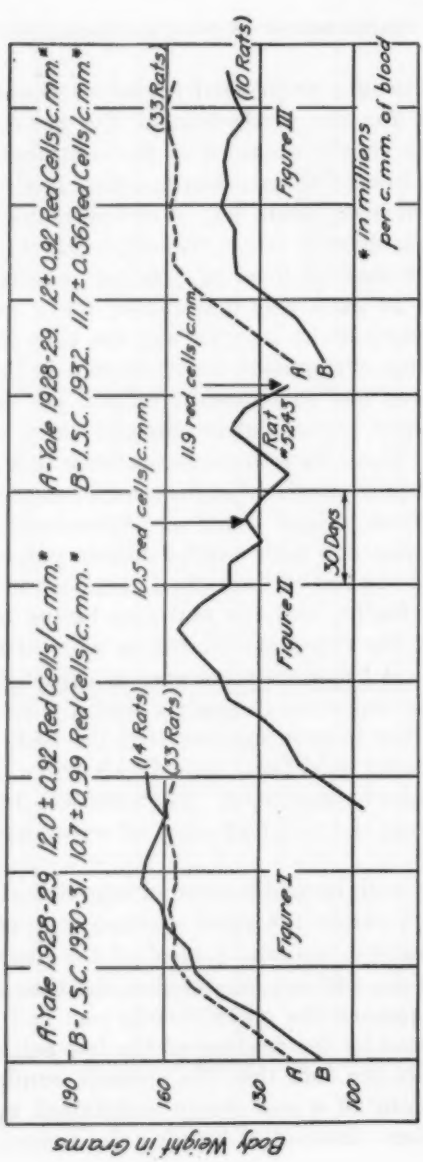

Chart 2 The relation between the length of time of maintenance of constant weight and the number of erythrocytes in the blood of rats fed a diet poor in salts.

adequate stock ration until a body weight of 120 gm. (the standard initial weight of the Yale animals) had been attained, the pre-experimental period was arbitrarily cut to 9 days. With this treatment, the average initial weight at the beginning of the experiment was only 104 gm. Furthermore, the rats grew only for 1 month and thereafter maintained a constant body weight for the remaining time (56 days). The average number of the erythrocytes in the blood of this group of rats was eleven and seven-tenths million per cubic millimeter (fig. III, chart 2), thus approximating the average number in the blood of the group studied in the Yale laboratory which had the same characteristic type of growth curve. The curves obtained in the two laboratories seem to indicate that a definite period of inhibited growth is more closely correlated with the severity of the induced polycythemia than is the level at which such inhibition occurs. It is to be emphasized at this point, however, that suppressed growth per se is not responsible for the polycythemic blood. For instance, retardation of growth brought about either by the limitation of food energy (Smith and Schultz, '30; Swanson and Smith, '32 a) or by the restriction of lysine (Gross, '34) is not accompanied by the marked increase in the number of erythrocytes discussed in the foregoing paragraphs. The retarded growth must be induced by a lack of the mineral salts in the diet if polycythemia is to occur.

From the above experiments, it appears that the investigator interested in the study of the physiological adjustments produced by the elimination of the dietary salts will find it necessary to study the reaction of his particular strain of animals to this specific nutritive deficiency. Furthermore, coprophagy must be prevented in so far as possible and utmost cleanliness of cages and accessories must be maintained if adequate suppression of growth is to occur. We have found that a very narrow margin exists between the quantity of inorganic materials which will restrict growth and the amount which will permit it.

Along with the characteristic suppression of growth, changes occurred in the general appearance of the rat. When the animal had developed a severe polycythemia it was decidedly marasmic. This was not true when the polycythemia was mild; the animal then looked nearly normal. It was surprising to note to what a slight degree the dietary deficiency was reflected in the appearance of the rat when polycythemia did not develop. When a definite polycythemia was present, the body was angular, the hair rough and often absent in certain parts of the body. The standing posture was high and angular. The rat frequently showed signs of extreme nervousness and hyperactivity. Evidence of a functional disturbance of the neuromuscular system occurred late in experimental history. Often the rat lost its sense of equilibrium, and in some instances, severe paralysis of legs and sphincters developed. The snouts were dirty and bloody. The eyes, ear, and paws became less and less pink and near the end of the experimental period were gray-white. The tail was dry and scaly. The leg bones and feet were long and large in proportion to the size of the animal. A severe diarrhea appeared almost immediately upon the administration of the low salt food and continued for approximately 3 weeks. Even though the intensity of the diarrhea subsided, the feces remained somewhat softer than normal throughout the entire experimental period.

At autopsy, there was extreme paucity of visceral fat. The cecum was often distended, and enlargement of the kidneys and adrenals was observed. Although the kidneys were frequently of a gray color with a mottled appearance, other visceral organs appeared normal. The lungs rarely showed atelectases or infection—a finding in harmony with the suggestion of Moïse and Smith ('30) that the active rat is less likely to develop bronchial obstruction and subsequent atelectasis than is the sluggish animal.

The restriction of growth, the change in body contour, and the distrophic growth of the long bones as well as the depletion of the skeleton of ash constituents were all revealed in

x-ray pictures of rats fed the low ash diet. Extreme osteoporosis, not rickets, was indicated, shown also by difficulty experienced in handling the animals without fracturing a leg bone. The molars were often loose and the incisors broken. There is a decided uniformity in the picture presented by the rats reared upon a dietary regimen characterized by nearly a complete absence of the inorganic salts and, so far as we are aware, it is not like any thus far described.[5]

CONCLUSIONS

1. Unless the growth impulse can be inhibited in the mature albino rat by the feeding of a salt-poor ration with casein as the protein, a polycythemia is not produced.

2. Constant weight following a short period of sub-normal growth must be maintained for at least 56 days in order to induce a pronounced increment in the number of red cells during the experimental interval.

3. The polycythemia is accompanied by a characteristic marasmic bodily state.

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[5] The extent of the deficiency in inorganic salts is indicated by data in the next paper of this series (Smith, A. H., and P. K. Smith. 1934. J. Biol. Chem., vol. 107, p. 681.

THE DERIVATION OF FACTORS FOR COMPUTING THE GASEOUS EXCHANGE AND THE HEAT PRODUCTION IN THE METABOLISM OF CASEIN BY THE ALBINO RAT [1]

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(Received for publication March 19, 1934)

In connection with recent experiments conducted in this laboratory on the specific dynamic effect of casein, the question arose as to what factors should be used in computing from the amounts of casein metabolized, as measured by the excretion of urinary nitrogen, the heat, CO_2 and the O_2 equivalents. The factors which are commonly employed for computing the respiratory exchange and the heat production of protein metabolism are those calculated by Loewy ('11) which represent the metabolism of meat protein in the dog. Loewy has also reported in the same publication somewhat different factors for milk protein which were calculated on the basis of work of Rubner (1898) and Rubner and Heubner (1898) with human beings. Since in the casein experiments cited above the albino rat served as the experimental animal, it was thought desirable to determine the factors to be used directly with these animals. The results of these endeavors are recorded below.

Nitrogen, carbon and energy balance experiments were conducted with a group of five rats, each weighing about 100 gm. and receiving 3.8 gm. of commercial casein daily.

[1] Publication authorized by the director of the Pennsylvania Experiment Station January 15, 1934, as technical paper no. 631.

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The casein used was found, on analysis, to contain 7.31 per cent moisture, 4.16 per cent ash, 13.18 per cent nitrogen, 47.24 per cent carbon, 0.54 per cent ether extract and 5.097 calories per gram.

The rats were kept in individual cages and were fed twice daily, receiving the experimental diet in two equal portions, for a period of 6 or 7 days. The first 3 or 4 days were preliminary, while the last 3 days constituted the excreta collection period. During the collection period the rats were kept in metabolism cages which were very similar to that described by Levine and Smith ('25), and the feces and urine were collected separately.

The urine and the feces were analyzed for total nitrogen, carbon and energy, the urine of each rat being analyzed separately. The quantities of feces collected for the individual rats were small, and for this reason they were composited, after air drying, into one sample for analysis.

The average results of the balances of matter and energy, and the derivation of the respiration and energy factors for casein, are shown in table 1.

The data of table 1 are for the most part self-explanatory. All the data in the upper part of the table are as experimentally determined. The values given for energy and carbon per 100 gm. of pure casein are based directly on the analysis of the casein used, assuming that the effects on these values of the small quantities of fat and carbohydrate contained in the casein are negligible. In fact, it was calculated, on the basis of the analysis of the material that the error involved by this assumption is less than 1 per cent. The values given for nitrogen, carbon and energy in feces and urine per 100 gm. of pure casein are computed from the determined values, in the upper part of the table, in proportion to the nitrogen content of the casein.

No experimental determinations were made of the intramolecular hydrogen or oxygen content in either the casein or the excreta, and these values were derived as indicated. The computation of the hydrogen and oxygen content of the

TABLE 1

Determination of factors for computing the respiratory exchange and the heat production in the metabolism of casein

	ENERGY	NITROGEN	CARBON	HYDROGEN (INTRAMOLECULAR)	OXYGEN (INTRAMOLECULAR)
	Calories	*Grams*	*Grams*	*Grams*	*Grams*
As determined					
Income, 3.8 gm. casein	19.367	0.501	1.795		
Outgo { feces	0.662	0.017	0.059		
Outgo { urine	3.056 ± 0.049	0.479 ± 0.006	0.240 ± 0.007		
Metabolizable	15.649		1.496		
Computed per 100 gm. pure casein					
Income	575.69[1]	15.70[2]	53.35[1]	7.00[3]	22.65[3]
Outgo { feces	20.75[2]	0.53[2]	1.85[2]	0.20[4]	0.83[4]
Outgo { urine	95.77[2]	15.01[2]	7.52[2]	2.51[4]	13.30[4]
Metabolizable	459.17		43.99	4.29	8.52
Intramolecular H_2O				1.07	8.52
Oxidized to CO_2 and H_2O			43.99	3.22	

O_2 required to produce $CO_2 = 43.99 \times 32/12$	= 117.31 gm.	
O_2 required to produce $H_2O = 3.22 \times 8$	= 25.76 gm.	
O_2 required in respiratory exchange	= 143.07 gm.	= 100.12 liters
CO_2 produced in respiratory exchange = 117.31 + 43.99	= 161.30 gm.	= 82.17 liters
Respiratory quotient	= 0.821	
Liters O_2 required per gram urinary N	= 100.12 ÷ 15.01 =	6.67
Liters CO_2 produced per gram urinary N	= 82.17 ÷ 15.01 =	5.47
Calories metabolized per gram urinary N	= 459.17 ÷ 15.01 =	30.59
Calories metabolized per liter O_2	= 459.17 ÷ 100.12 =	4.586

[1] As determined on the basis of the water-free and ash-free substance.

[2] From Mathews ('30, p. 117).

[3] Calculated in relation to the nitrogen content of the casein.

[4] Calculated from the figures of Loewy ('11) for the excretion products of meat in relation to the nitrogen content of the material. According to these calculations for each gram of nitrogen ingested, 0.013 gm. of hydrogen and 0.53 gm. of oxygen appear in the feces and 0.160 gm. of hydrogen and 0.847 gm. of oxygen appear in the urine.

excreta probably involves a slight error, inasmuch as this computation assumes that the excretion products, in relation to nitrogen, are the same as those of meat proteins in the dog. However, the writers are not aware of any better data than those used upon which to base this computation.

Of the total nitrogen ingested, 3.4 per cent appeared in the feces and 95.6 per cent appeared in the urine, leaving a positive balance of only 1 per cent.

Of the total calories ingested, 3.4 per cent appeared in the feces and 15.8 per cent appeared in the urine. In other words, 96.6 per cent of the calories of the casein were absorbed and only 80.8 per cent were metabolized. It should perhaps be stated here that the energy of the urine was corrected, as usual, to nitrogen equilibrium, the correction being very small on account of the very small positive nitrogen balance.

Calculated on the basis of pure casein, 3.6 per cent of the total calories appear in the feces and 16.6 per cent in the urine, 96.4 per cent being absorbed and 79.8 per cent metabolized.

The values obtained for O_2 required, and for CO_2 and heat produced, in the metabolism of protein, as casein, are 6.67 liters, 5.47 liters, and 30.59 Calories, respectively, per gram of urinary nitrogen. The respiratory quotient is 0.821, and the calorific value of respiratory O_2 is 4.586 Calories per liter. Loewy's ('11) factors for milk protein are 5.0 liters O_2, 4.6 liters CO_2 and 27.0 Calories per gram of nitrogen in the urine. His factors for meat protein are 5.94 liters O^2, 4.75 liters CO_2 and 26.51 Calories per gram of urinary nitrogen.

Rapport ('24) has calculated the following factors for gelatin: 1 gm. of N in the urine = 4.70 liters O_2, 3.92 liters CO_2 and 22.08 Calories.

Rapport's observation that the calculation of the metabolism of proteins other than meat on the basis of factors for meat protein involves a relatively small error in the total heat production is confirmed by the writers. This can be explained by the fact that the calorific value of a liter of re-

spiratory O_2 in the metabolism of protein does not differ much from the calorific value of O_2 in the metabolism of fat, or of a mixture of fat and carbohydrate. It is well to point out, however, that the percentage contribution of the protein metabolism to the total heat production may be appreciably affected if the calories per gram of urinary nitrogen for a particular protein differ considerably from the corresponding value for meat.

The factors for casein determined in table 1 differ markedly from Loewy's factors for milk protein. In view of the fact that Loewy's figures for CO_2 and O_2 give a respiratory quotient as high as 0.92, the writers feel justified in suspecting that some of Loewy's figures are in error. This suspicion is strengthened by the fact that by dividing the calorific value of a liter of respiratory O_2 (4.66 Calories), given by Loewy, into the calories per gram of urinary nitrogen (27.0) the result obtained, which should represent liters of O_2 per gram of urinary nitrogen, is 5.8 instead of the figure 5.0 which appeared in Loewy's report.

SUMMARY

Balances of nitrogen, carbon and energy were determined with five albino rats receiving casein exclusively in quantities sufficient approximately to meet the energy requirements.

Of the total calories of the casein ingested 96.6 per cent were found to be digested, and 80.8 per cent were found to be metabolizable.

The following factors were determined for computing the gaseous exchange and the heat production in the metabolism of casein: 1 gram of urinary nitrogen = 6.67 liters of respiratory O_2, 5.47 liters of CO_2 and 30.59 Calories.

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THE EFFECT OF VITAMIN A DEFICIENCY ON THE CONCENTRATION OF THE BLOOD LIPIDS OF ALBINO RATS

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The work in this paper is an investigation of fatty acids, lecithin, and cholesterol of the blood of rats when the diet is deficient in vitamin A.

Fat metabolism in avitaminosis has been studied quite extensively by Kazuo Asada ('23 a). He used young rats for his experimental animals, and fed them a vitamin free diet, consisting of wheat protein, starch and dried carrots autoclaved, a salt mixture and distilled water. In his first published report Asada found an increase of blood fat accompanied by a decrease of the total fat of the whole body. It was necessary, therefore, to assume an increased fat combustion and a decreased fat storage. These results about the behavior of fats in avitaminosis led to the knowledge "That there was a disturbance in the movement of the fat, and an increased transport of the body and food fats to the places of combustion."

In Asada's ('23 b) second report emphasis is placed upon the blood as a means of fat transportation. The tabulated results show that "The amount of fat in the blood of avitaminotic animals is somewhat higher than that of normal animals. The cholesterol content of the blood goes parallel with the total fat. . . . In the last stages of avitaminosis there is a decrease in the blood fat which is due to the fact that the

fat depots are exhausted and do not give any more to the blood." In this third report Asada ('23 c) again announces that, the fat and cholesterol content of the bodies of avitaminotic animals decrease while the concentration of the fat and cholesterol of the blood increase.

Fukunda ('30) reported on the change of the "Fat content of the blood in animals fed with a vitamin-poor diet." "In various avitaminoses with animals and birds there is always an increase in the fat content of the blood, the increase being sometimes appreciable even before the manifestations of the symptoms. . . . The character of increased fat is different with different types of avitaminosis. . . . In animals affected with avitaminosis the fat metabolism is deranged."

Wendt ('28), in summarizing his experiments on "Lipid metabolism studies on starving animals," states that, "The lipid phosphorus and cholesterol in the peripheral blood of fasting dogs increase the first few days of the fast and then decrease to sub-normal values as the fasting continues."

Collazo and Bosch ('23) used guinea pigs and dogs for their experimental animals. They concluded that in the course of avitaminosis there was an increase in the total blood fat which decreased at the end of the disease without reaching the normal value. The cholesterol content of the blood went parallel with the fat values. The phosphatide content of the blood was normal at the beginning of avitaminosis and sometimes decreased in the later phases of the disease.

EXPERIMENTAL

Albino rats from 50 to 75 days of age were used in this experiment. The animals were arranged in litter mate triads of the same sex, and as nearly comparable in weight as possible. When weight dissimilarity was unavoidable the heaviest animal was chosen for the vitamin A deficient member of the triad, the lightest one was made positive control and the one of medium weight was chosen for the calorie control, which received only as much food per gram of body weight as was consumed by the vitamin A deficient animal. The positive

controls received food ad libitum. After the appearance of conjunctivitis, the calorie and positive controls were given four drops (120 mg.) of cod liver oil daily throughout the entire experiment.

Some of the mothers of the experimental animals were bred while they were being fed on stock diet no. 2 (Sure, '28). The formula for this diet is, ground whole wheat 2640 gm., whole milk powder 1308 gm. and 52 gm. of NaCl. The nurslings were fed by the mothers on this diet, and when needed for experiments were taken directly from stock diet no. 2 and placed on the vitamin A deficient diet no. 1034 (Sure, et al, '33), which consisted of 20 per cent hot alcohol extracted casein, 10 per cent Northwestern yeast, 4 per cent salts no. 185 (McCollum and Simmonds, '17), and 66 per cent dextrin. The mixture was irradiated with a Cooper Hewitt ultraviolet lamp for 30 minutes.

The mothers of the remaining triads were nourished on stock diet no. 1 (Sure, '26) which consisted of 27 per cent whole wheat, 26 per cent rolled oats, 25 per cent yellow corn meal, 15 per cent linseed oil meal, 5 per cent commercial casein, 1 per cent cod liver oil, 0.5 per cent NaCl, and 0.5 per cent $CaCO_3$, with a liberal supply of whole milk. The vitamin A deficient animals of the triads fed on stock diet no. 1 were more resistant to the onset of ophthalmia and lived longer than those whose mothers were fed on stock diet no. 2 (Sure, '28) during gestation and lactation. They evidently had a storage of food constituents, including vitamins, which enabled them to combat a vitamin A deficient diet longer than those fed on stock diet no. 2.

In this study ten triads, or thirty animals, were used, fifteen for the determination of fatty acids and lecithin, and fifteen for cholesterol. Twenty-four of the thirty animals were females. Although the appearance of ophthalmia was taken as the definite indication of vitamin A deficiency, evidence of the pathological condition was first noted with the continuous occurrence of cornified cells in the vaginal smears of the females. This phenomenon appeared from 7 to 15 days be-

fore the symptoms of ophthalmia could be detected. Evans ('28) made a very comprehensive study of the keratinization of uterine epithelium and found the occurrence sufficiently constant to be used as a means of identification of vitamin A deficiency. Aberle ('33) also states that cornification of cells invariably precedes other symptoms of vitamin A avitaminosis.

As soon as the animals were placed on a vitamin A deficient diet, ½ ml. of peripheral blood was taken once per week from each animal of the fatty acid-lecithin lot, and a similar amount of blood from those from which cholesterol was to be obtained. The animals were always fasted 16 hours before bleeding.

The fatty acids were estimated according to the method of Smith and Kik ('33). The inorganic phosphorus from whole blood from which lecithin was computed, was determined by the Fiske and Subbarow method ('25) modified by F. C. Koch ('34), cholesterol was estimated colorimetrically by use of the Liebermann-Burchard reaction. A complete description of the process is given by Bloor, Pelkan and Allen ('22).

Until the calorie and positive control animals received cod liver oil the dietary regime was constant for all.

As conjunctivitis was taken as the definite symptom of a pathological condition, somewhat similar in all the animals, the amount of lipids determined from the blood at this particular stage in the disease was used as a basis from which to calculate the means when all the animals were fed on a diet deficient in vitamin A.

RESULTS

Table 1 gives the mean number of milligrams of fatty acids, lecithin and cholesterol in 100 ml. of the blood of the three groups combined, before the administration of cod liver oil to the controls.

After cod liver oil was given to the control animals, the purpose of the triad arrangement becomes more significant.

In the tables the vitamin A deficient animals will be designated P, the calorie controls as R, and the positive controls as C.

TABLE 1

Mean amounts of fatty acids, cholesterol and lecithin in 100 ml. blood previous to the administration of cod liver oil. Seven blood analyses were made for fatty acids and lecithin and six analyses for cholesterol.

Vitamin A deficient animals

NUMBER OF ANIMALS	FATTY ACIDS	NUMBER OF ANIMALS	CHOLESTEROL	NUMBER OF ANIMALS	LECITHIN
	M PE		M PE		M PE
	mg.		*mg.*		*mg.*
3	306.3 ± 3.69			3	285.9 ± 2.03
6	334.4 ± 11.60	3	88.3 ± 2.94	6	306.7 ± 6.74
6	318.7 ± 6.73	3	88.1 ± 6.03	6	306.9 ± 5.52
12	297.8 ± 4.45	3	126.8 ± 3.37	12	308.9 ± 4.44
12	292.1 ± 5.18	8	100.1 ± 5.83	12	289.3 ± 6.73
12	323.8 ± 7.62	11	114.5 ± 3.24	12	289.0 ± 5.48
12	306.0 ± 5.13	12	126.5 ± 3.72	12	283.0 ± 3.35
	Cod liver oil given at this point				

M, mean. PE, probable error.

Table 2 gives the mean number of milligrams of fatty acids, cholesterol and lecithin per 100 ml. of blood, at each bleeding, for the groups P, R, C, after the two controls have received therapeutic treatment.

Fatty acids and lecithin were determined from the same blood sample. When cholesterol was extracted first, then fatty acids and finally the lecithin from the same blood sample, fatty acids and lecithin were invariably less than when extractions were made from duplicate samples previous to the extraction of cholesterol. For this reason a different set of animals was used for cholesterol determinations.

TABLE 2

The mean amounts of fatty acids, cholesterol and lecithin in 100 ml. blood for the individual bleedings of each group in the triads after the administration of cod liver oil to the controls

NUMBER OF ANIMALS	P		R		C	
	M	PE	M	PE	M	PE
Fatty acids						
	mg.		*mg.*		*mg.*	
1	248.4	± 17.28	266.1	± 17.28	248.4	± 17.28
2	330.4	± 12.21	297.0	± 12.21	297.0	± 12.21
3	328.0	± 9.98	288.9	± 9.98	294.1	± 9.98
5	323.6	± 7.73	299.1	± 7.73	270.9	± 7.73
5	279.1	± 7.73	318.1	± 7.73	273.9	± 7.73
Cholesterol						
3	124.1	± 3.52	116.1	± 3.52	108.9	± 3.52
4	119.2	± 2.49	105.1	± 2.49	107.2	± 2.49
4	123.1	± 2.49	115.8	± 2.49	111.4	± 2.23
5	120.9	± 2.23	118.7	± 2.23	107.9	± 2.23
5	122.5	± 2.23	119.8	± 2.23	104.6	± 2.23
5	104.3	± 2.23	121.3	± 2.23	112.1	± 2.23
Lecithin						
1	285.2	± 11.16	280.7	± 11.16	254.0	± 11.16
2	299.8	± 7.94	292.2	± 7.94	282.0	± 7.94
3	324.4	± 6.48	280.1	± 6.48	276.2	± 6.48
5	295.1	± 5.02	280.1	± 5.02	272.3	± 5.02
5	325.2	± 5.02	277.4	± 5.02	267.0	± 5.02

P, vitamin deficient animal. M, mean. R, calorie control. PE, probable error. C, positive control.

Table 3 gives a comparison between groups at each bleeding, after the administration of cod liver oil to the controls. It shows the variations in the amounts of the three blood lipids for the groups P, R, and C.

The differences in fatty acids between P and C gradually increase until the fourth bleeding when the excess becomes highly significant, P being the greater. At the last bleeding the pathological animals are in a moribund condition and the blood fatty acids are similar in amount to those of the positive controls.

TABLE 3

Comparison between groups at each bleeding after administration of cod liver oil to the controls

BLOOD SAMPLE	FATTY ACIDS			CHOLESTEROL			LECITHIN		
	Group	Mean amount	$\frac{M_D}{PE_{MD}}$	Group	Mean amount	$\frac{M_D}{PE_{MD}}$	Group	Mean amount	$\frac{M_D}{PE_{MD}}$
		mg.			*mg.*			*mg.*	
1st	R	266.1	1.00	P	124.1	1.29	P	285.2	0.40
	P	248.4		R	116.1		R	280.7	
	R	266.1	1.00	P	124.1	2.46	P	285.2	2.79
	C	248.4		C	108.9		C	254.0	
	P	248.4	0.00	R	116.1	1.16	R	280.7	2.39
	C	248.4		C	108.9		C	254.0	
2nd	P	330.4	1.93	P	119.2	2.63	P	299.8	0.68
	R	297.0		R	105.1		R	292.2	
	P	330.4	1.93	P	119.2	2.24	P	299.8	1.59
	C	297.0		C	107.2		C	282.0	
	C	297.0	0.00	C	107.2	0.37	R	292.2	0.91
	R	297.0		R	105.1		C	282.0	
3rd	P	328.0	2.77	P	123.1	1.30	P	324.4	4.86
	R	288.9		R	115.8		R	280.1	
	P	328.0	2.40	P	123.1	1.40	P	324.4	5.29
	C	294.1		C	111.4		C	276.2	
	C	288.9	0.37	R	115.8	0.57	R	280.1	0.43
	R	294.1		C	111.4		C	276.2	
4th	P	323.6	2.26	P	120.9	0.40	P	295.1	2.12
	R	299.1		R	118.7		R	280.1	
	P	323.6	4.83	P	120.9	2.40	P	295.1	3.23
	C	270.9		C	107.9		C	272.3	
	R	299.1	2.59	R	118.7	1.99	R	280.1	1.00
	C	270.9		C	107.9		C	272.3	
5th	R	318.1	3.58	P	122.5	0.50	P	325.2	6.77
	P	279.1		R	119.8		R	277.4	
	R	318.1	4.05	P	122.5	3.30	P	325.2	8.24
	C	273.9		C	104.6		C	267.0	
	P	279.1	0.48	R	119.8	2.80	R	277.4	1.47
	C	273.9		C	104.6		C	267.0	
6th				R	121.3	3.30			
				P	103.4				
				C	112.1	1.42			
				P	103.4				
				R	121.3	1.69			
				C	112.1				

M, mean. M_D, mean difference. PE_{MD}, probable error of mean difference. $\frac{MD}{PE_{DM}}$, significant ratio. 2.5 to 2.8 approaching significance: 2.8 to 3.0 significance: above 3.0 is high significance.

P significantly exceeds R in fatty acids at the third bleeding. At the fourth the excess is small. At the last bleeding the excess of R over P is highly significant. The excess of R over C approaches significance in the bleeding before the last and becomes highly significant at the last.

A similar comparison between groups at each bleeding to determine differences in cholesterol, shows that P exceeds C significantly at the fifth bleeding, which is the one before the last. In the first four bleedings there is no uniform gradation of differences. At the last bleeding, C is slightly in excess of P. The excess of P over R for cholesterol approaches significance at the second bleeding and after that diminishes to insignificance. At the last bleeding the difference is inverted and R significantly exceeds P. The cholesterol content of the blood of the R group is slightly in excess of C from the third bleeding. At the last bleeding, P is the least of the three groups.

Because the concentration of the fatty acids and cholesterol of the blood of the vitamin A deficient animals became significantly less when the animals were in a moribund condition and the lecithin remained higher than that of either of the controls until the end of the experiment, fatty acids and cholesterol were placed consecutively in the tables, notwithstanding the fact that they were obtained from different blood samples.

The comparison between groups at each bleeding for lecithin, after the controls had received therapeutic treatment, showed that P exceeds R significantly at the third and fifth bleedings, but the differences are not significant in the other three cases. P exceeds C significantly in all the bleedings except the second. In contrast to the results obtained for fatty acids and cholesterol, the lecithin content of the blood of the vitamin A deficient animals at the last bleeding shows differences in excess of the two controls which are highly significant.

These results are very similar to those obtained by Asada ('23 a, b, c). He states that Susuki explained the decrease

in fat oxidation in avitaminosis as due to the lack of vitamin A, which oxidizes fat. When there is a decrease in fat oxidation the protoplasmic cells do not absorb the blood fat with the same speed, and the fat remains in the blood at a higher level. Later in avitaminosis, the blood fat decreases perhaps without reaching the normal value, due to the fact that the fat depots are exhausted and do not give any more to the blood. Asada, Susuki (quoted by Asada) and Collazo and Bosch ('23) showed that with avitaminotic animals, blood fat and cholesterol increased. The amount of increase depended upon the phase of the disease and especially upon the intensity with which the fat depots were emptied.

Table 1, on page 679, gives the mean amounts of fatty acids, cholesterol and lecithin at each bleeding for all the animals before the administration of cod liver oil to the controls. Table 2, on page 680, records the mean amounts of the three lipid constituents, at each bleeding, for the groups, P, R, and C after the controls received therapeutic treatment.

Table 3, which is the result of a more detailed statistical analysis, shows that the blood fatty acids and cholesterol of the vitamin A deficient animals exceed those of the control groups until the last bleeding. Then an inversion occurs. During the last week of the experiment the vitamin A deficient animals were very ill, consumed little food, and lost weight rapidly. It is reasonable that at this stage of the disease, the fat depots would be depleted of their supply, and the amount of fat furnished to the blood relatively decreased.

Wendt ('28) worked with starving animals and concluded from experimental results that during the first few days of starvation much lipid material came from the transformed fatty tissue and was liberated into the blood stream.

In this experiment the calorie controls received the same amount of food per gram of body weight as was consumed by the vitamin A deficient animal of the same triad. During the last week of the experiment the calorie controls were inadequately nourished and, being healthy animals, had to depend upon endogenous reserves in order to carry on normal

body processes. The amounts of fatty acids and cholesterol in the blood of the animals increased on account of the transportation of these substances from the fat depots to the places of combustion.

The amount of lecithin in the blood of the vitamin A deficient animals remained in excess of the two controls until the end of the experiment. Degkwitz ('31) has made a recent review of the literature on lecithin/cholesterol balance. He finds that results show an essential antagonism between these two substances as regards cellular physiology.

Henrickson ('29) studied "General cell degeneration as a result of vitamin A deficiency" and found that rats in advanced stages of avitaminosis A showed general cell degeneration in all the organs. No postmortem examinations of tissues were made in this study and, therefore, no comparisons are justified except in the cases of continuous appearance of cornified cells in the vaginal smears and xerophthalmia which are cited in the order of their occurrence.

A comparison between groups at each bleeding after the administration of cod liver oil to the controls showed significant difference as avitaminosis advanced (table 3). In order to check these results the differences between groups, triads, and individual bleedings were obtained by 'analysis of variance' (Fisher, '30).

In determining the significance of various contributors to variance, interpretations were made by the use of 'F' (Snedecor, '34). This analysis shows a highly significant contribution of variance in the groups (P, R, and C) for fatty acids, cholesterol and lecithin. The contribution of variance due to bleedings was not significant for lecithin and cholesterol, but did show significance for fatty acids. The variance due to the triads was highly significant for lecithin and cholesterol. The triads, the mothers of which were fed on stock diet no. 1, had a higher lecithin and cholesterol concentration in the blood than those whose subsistence could be traced back to stock diet no. 2.

The precipitous fall in fatty acids and cholesterol when the vitamin A deficient animals were in a moribund condition was characteristic of this avitaminosis and independent of dietary regime.

The contribution of variance due to sex was not significant for any of the three blood lipids studied in this experiment.

SUMMARY

1. A diet deficient in vitamin A produced a continuous occurrence of cornified cells in the vaginal smears of the female albino rats, from 7 to 15 days before the appearance of ophthalmia.

2. After the administration of cod liver oil to calorie and positive controls the fatty acid and cholesterol content of the blood of the vitamin A deficient animals exceeded that of the two controls. At the bleeding before the last, the fatty acids and cholesterol of the vitamin A deficient animals significantly exceeded the calorie and positive controls. An inversion was evident at the last bleeding. The calorie controls then showed a significant excess of fatty acids and cholesterol over the vitamin A deficient animals.

3. From the third to the last bleedings, inclusive, the lecithin content of the blood of the vitamin A deficient animals was significantly higher than that of either of the controls, with the exception of the fourth bleeding, when the difference between vitamin A deficient animals and calorie controls was only slightly significant.

4. In the comparison between groups at each bleeding after the administration of cod liver oil, analysis of variance was applied to test for significant differences between groups, triads and individual bleedings. This analysis proved a highly significant difference between groups for fatty acids, cholesterol and lecithin. The differences between the individual bleedings were found to be significant only for the fatty acids.

5. The differences between the triads were significant for lecithin and cholesterol. The reason for these variations was traced back to the nutritional background of the animal.

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THE RELATION BETWEEN THE ANTIRACHITIC FACTOR AND THE WEIGHT OF THE GALL BLADDER AND CONTENTS OF THE CHICKEN [1]

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Brief reference (Russell and Chichester, '31) has already been made regarding the observation that the average weight of gall bladders and contents of chicks receiving a ration deficient in the antirachitic factor is greater than that of normal chicks. Loeffler ('28) has called attention to the enlargement of the gall bladder of the mouse when bile acids were fed, but no mention was made of the presence or absence of an antirachitic substance in the diet. This paper is a presentation of further data concerning the observation previously reported.

Experiment 1

Procedure. The four groups of White Wyandotte chicks used in this study were fed a basal ration, with or without a supplement, as indicated in column 1 of table 1, which consisted of 47 per cent ground yellow corn, 20 per cent wheat middlings, 15 per cent wheat bran, 5 per cent dried buttermilk, 9 per cent meat scrap (55 per cent protein), 3 per cent ground oyster shell and 1 per cent sodium chloride. Cod liver oil was administered by capsule, five or six times per week, in an amount based upon the weekly consumption of

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Agricultural Biochemistry.

a ration containing 1 per cent of the oil (Card and Kirkpatrick, '18), existing data on food consumption by another heavy breed, Rhode Island Reds, being used for the calculation. Irradiated ergosterol in corn oil and corn oil alone were administered in the same manner as the cod liver oil and in the same volume. As indicated in table 1, the number of rat units of the antirachitic factor in the form of irradiated ergosterol was six times that fed as cod liver oil. The chicks

TABLE 1

Summary of data obtained at 7.5 weeks of age

GROUP NUMBER AND TREATMENT	NUMBER OF BIRDS	AVERAGE BODY WEIGHT AT 7.5 WEEKS OF AGE	AVERAGE WEIGHT OF GALL BLADDERS AND CONTENTS	MAXIMUM AND MINIMUM WEIGHTS OF GALL BLADDERS AND CONTENTS	COEFFICIENT OF VARIATION	BONE ASH
		gm.	*mg.*	*mg.*	*per cent*	*per cent*
1 Cod liver oil 1 per cent level	36	557	294	564 70	39	47.93
2 Irradiated ergosterol in corn oil. Equivalent to 6 per cent cod liver oil	31	474	543	1027 160	46	44.45
3 Corn oil. Same volume as groups 1 and 2	12	371	1195	3827 421	74	40.07
4 Basal	29	337	699	2270 128	71	39.69

were killed by decapitation, and the gall bladders and contents dissected out and weighed in a weighing bottle. Bone ash was determined by a method previously described (Russell and Massengale, '28).

Results and discussion. Upon the basis of bone ash percentage and body weight, group 1 (table 1) is classed as normal for the breed at the age of killing. The coefficients of variation indicate that there is considerable variation of values within a group, but the average weight of the gall bladders and contents of group 1, the normal group, is

significantly lower than those of the irradiated ergosterol, corn oil, and basal groups. The gall bladders of the deficient birds were turgid with bile in practically every case, whereas this condition occurred rarely in the protected groups. When those of the deficient groups were not filled with bile, they had a stretched, flaccid appearance. In contrast, those of the cod liver oil groups were usually in a contracted state. The bone ash percentage of the irradiated ergosterol group lies between that of the normal and those of the groups deprived of the factor and indicates incomplete protection against leg weakness. It is of interest that the average weight of the gall bladders of the irradiated ergosterol group also lies between the values for the two groups just mentioned. As stated above, the antirachitic potency of the irradiated ergosterol is six times that of cod liver oil on the basis of a rat assay. These facts suggest that gall bladder size in the chicken is associated with the effectiveness of the factor in promoting bone formation, and not with the number of rat units consumed.

The basal group did not receive an oil supplement, but the other groups were given an amount of oil per week, corn oil or cod liver oil, which increased from 0.5 cc. during the first week to 3.6 cc. during the last week of the experiment. Although the average weight for the corn oil group is higher than the others, the difference between the average weights of gall bladders of this and the basal group does not have much statistical significance, because of the high coefficients of variation and probable errors involved, and the small population of the corn oil group. The observation suggests further study of the effect of fat in the ration, with and without the antirachitic factor, on the size of the gall bladder.

Experiment 2

Ivy and Oldberg ('28) have shown that the injection of hydrochloric acid into the duodenum of the dog under barbital anesthesia caused the gall bladder to contract. Hence the question was raised whether there was a difference in H-ion

concentration of the duodenal contents of birds with and without the antirachitic factor, which might account for the greater bile content of the bladders of birds deprived of the factor. Also it was of interest to determine whether there were differences in total solids, ash, and calcium contents of the biles from the two groups.

Procedure. Duodenal contents were available from two groups of chicks at 8 weeks and bile at 5, 8, and 11 weeks of age. Both groups were fed the basal ration described under experiment 1, but group 1 was protected against leg weakness by the incorporation of 1 per cent cod liver oil in the ration, whereas group 2 was deprived of the antirachitic factor. Immediately after killing, the duodenal loop was removed and the H-ion concentration of the contents determined by means of the quinhydrone electrode (Cullen and Biilman, '25). The bile from each group was pooled and total solids were determined at 98° to 100°, the ash by destruction of organic matter in an electric muffle, and calcium of the ash volumetrically (Official Methods, '30).

RESULTS AND DISCUSSION

The group which received cod liver oil in the ration was adequately protected against leg weakness, but the basal group had developed severe leg weakness at 8 weeks of age. Although gall bladder weights were not obtained, as was done previously, the average volume of bile from the basal group, 0.41 cc., was more than twice that, 0.16 cc., from the protected group. The gall bladders of the deficient birds were markedly enlarged and turgid with bile as compared with the cod liver oil group, as was also observed in experiment 1.

The average pH of the duodenal contents of the basal group was 6.36 and of the protected group 6.46 at 8 weeks of age. This close agreement shows that H-ion concentration is not a factor which controls the amount of bile in the bladder under these nutritional conditions.

The total solids of the bile of birds which had received the antirachitic factor was practically constant throughout the

experiment, table 2, whereas the value for the basal group increased with age. The percentage of ash was found to fluctuate in the protected group, but a tendency to increase with age is again apparent in the deficient birds. Calcium expressed as mg. per 100 gm. of bile was 25 to 35 per cent lower in the basal as compared with the protected group, but the average volume of bile of the basal group at 8 weeks of age was of the order of twice that of the normal group. Hence

TABLE 2

Total solids, ash, and calcium content of pooled gall bladder bile

GROUP NO., SUPPLEMENT AND AGE OF KILLING	NUMBER OF BIRDS	TOTAL SOLIDS	ASH	CALCIUM
		per cent	*per cent*	*mg./100 gm. of bile*
1 1 per cent cod liver oil. Protective				
5 weeks	15	19.45	2.02	63.6
8 weeks	12	19.55	2.13	61.1
11 weeks	12	19.30	1.98	61.6
2 Basal				
5 weeks	15	18.45	1.91	46.0
8 weeks	12	20.50	2.20	39.6
11 weeks	12	21.65	2.30	45.3

although the concentration of calcium was lower in the bladder bile of the basal group, the total calcium in the bile of these bladders is probably greater than that in the bladders of the protected group. Whether more calcium goes into the intestine from the deficient bird than from the normal is not known, but would depend upon the frequency of the emptying of the bladder. If the enlarged gall bladders are indicative of greater excretion of bile into the intestine, the bile may be an important excretory route for calcium in the leg weakness condition.

SUMMARY

1. The average weight of the gall bladders and contents of a group of chicks deprived of the antirachitic factor was more than twice that of a group provided with an adequate amount of the factor.

2. When a group of chicks was only partially protected against leg weakness, as indicated by the percentage bone ash, the average weight of the gall bladders was intermediate between the values observed for a normal group and for one deprived of the factor.

3. The average H-ion concentration of the duodenal contents was essentially the same whether the groups had an adequate amount or were deprived of the antirachitic factor, and is apparently not related to the retention of bile by the gall bladder.

4. Total solids, ash and calcium were determined in the bile at 8 weeks of age.

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THE BASAL METABOLISM OF EUROPEAN WOMEN IN SOUTH INDIA AND THE EFFECT OF CHANGE OF CLIMATE ON EUROPEAN AND SOUTH INDIAN WOMEN

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FOUR FIGURES

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In presenting their data on the metabolism of south Indian women, Mason and Benedict ('31) suggested possible causes other than race which might be found to be factors in the low metabolism of this group. Among these causes was the factor of climate. If the tropical climate as such affects human metabolism that effect should certainly be apparent in the conditions prevailing in Madras. The city of Madras, lying on the southeast coast of India, 13° 4″ north of the equator at an altitude of 22 feet, has a mean annual temperature of 82.2° F., a relative humidity of 72 per cent, and a barometric pressure of 758.0 mm. The seasonal variation is slight, the highest monthly maximum temperature, 98.2° F., occurring in June, the lowest, 83.7°, in December. The lowest monthly minimum, 67.5°, occurs in January, the highest, 80.8°, in May. The monthly variations in humidity are from 62 per cent in June to 79 per cent in the rainy season in November. The barometer shows pressure variations of from 754.3 mm. in June to 761.8 mm. in January.

[1] This study was made possible by the loan of metabolism apparatus and by financial assistance from the Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.

Apart from a study of the races which are native to Madras, in whom the climatic factor may be obscured by other factors, such as activity or dietary habits or race itself, the effect of the Madras climate may be studied either by finding whether the members of white races residing in Madras show a different metabolism from those of the same race in temperate climates, or, better, by studying the effect of change of climate on the same individuals, both European and Indian, as they move their residence from one country to another. Both methods have been used in the experiments reported here.

SUBJECTS

Thirty-four European women, residents of Madras or its immediate environs, were measured. Table 1 shows their ages, race and physical measurements together with data concerning their association with the tropics. There was a wide range of age and considerable difference in configuration. The total length of residence in the tropics varied from 2½ months to 31 years, but the stays of longer duration had been interrupted by periodic furloughs. Of these thirty-four European women measured in Madras nine were studied more extensively for the effect of change of climate. In addition, measurements were secured on three of their Indian colleagues who went abroad to study.[2]

[2] The author is very grateful to Dr. F. G. Benedict for making many of the arrangements and to the physiologists and doctors in various places who have made measurements on these subjects in temperate environments. These are: members of the staff of the Nutrition Laboratory in Boston; Prof. Abby H. Turner, of Mount Holyoke College; Dr. Douglas Robertson, of the Middlesex Hospital Medical School, London; Prof. Jessie H. Rideout and Prof. C. C. Benson, of the University of Toronto; Dr. Paul Roth, of the Battle Creek Sanitarium; Dr. E. F. DuBois, of the Cornell University Medical College; Prof. Grace MacLeod, of Columbia University; Dr. L. H. Newburgh, of the University of Michigan; and Dr. Garfield Thomas, of the General Hospital, Birmingham, England.

I wish also to thank my two colleagues who made the measurements on me in Madras, Miss Beatrice S. Cosmey and Dr. Mariam P. Oommen.

TABLE 1

Physical characteristics and other data concerning European women in Madras

NUMBER AND SUBJECT		RACE	AGE	TOTAL TIME IN INDIA		TIME SINCE LAST ARRIVAL IN INDIA		WEIGHT	HEIGHT	SITTING HEIGHT	PELIDISI [1]
			Years	*Years*	*Months*	*Years*	*Months*	*kg.*	*cm.*	*cm.*	
1	W.D.	English	20	8		1		56.4	159	84.5	97
2	S.H.	Danish	21	9	6	3		56.2	160	83.0	99
3	G.E.S.	American	23		4		4	54.0	167	88.5	92
4	G.G.	Scotch	24	5	5		5	59.6	165	88.0	96
5	M.S.	English	25	1	1	1	1	50.3	175	91.5	86
6	M.H.	English	25		2½		2½	50.8	165	88.5	90
7	E.L.C.	Canadian	26		7		7	45.8	154	86.0	90
8	K.S.	English	27	1		1		45.8	161	85.0	91
9	B.S.C.	American	28	1	8	1	8	59.9	157	85.0	99
10	D.H.	English	29	3		3		55.4	170	91.0	90
11	M.R.B.	English	29	1	8	1	8	55.1	159	83.0	99
12	A.M.P.	Scotch	30		5½		5½	47.6	156	83.0	94
13	H.H.	Scotch	31		5½		5½	65.0	162	86.5	100
14	E.S.	English	31	5		5		49.9	162	83.0	96
15	D.E.H.	English	32	6		1	6	58.1	168	88.0	95
16	M.P.	English	32	5		5		50.3	170	89.5	88
17	E.D.M.	American	32	9		1	9	47.7	169	86.0	91
18	E.P.	Danish	32	1	9	1	9	54.3	175	88.5	92
19	M.D.B.	American	36	10		5		52.2	160	85.5	94
20	S.C.F.	American	37	19		1	8	50.3	162	88.5	89
21	O.E.J.	American	38	8		2	2	44.9	159	80.0	96
22	O.M.S.	American	38	10			9	50.2	159	84.0	95
23	E.J.	English	39	10		1	1	50.5	158	84.0	95
24	A.M.V.	English	40	13		1	6	68.0	170	89.0	99
25	K.N.B.	English	41	16		2	2	57.4	167	87.5	94
26	E.M.C.	American	41	11			11	52.4	165	85.5	94
27	I.McN.	Scotch	42	11			11	71.1	169	89.5	99
28	M.L.D.	English	44	18		1	6	53.5	160	84.5	96
29	A.G.G.	Scotch	45	1		1		48.5	161	85.0	92
30	J.E.B.	American	46	12			4	53.1	156	84.5	95
31	G.E.C.	American	47	31		2	7	70.3	168	87.0	102
32	A.V.D.	American	50	23			6	58.9	166	85.0	99
33	A.G.S.	American	54	1	8	1	8	56.7	154	79.5	103
34	E.McD.	English	54	11		1	9	57.4	164	84.0	99
Average—34 Europeans			35					54.6	163	85.9	95
Average—54 Indians [2]			21					45.0	154	78.5	97.5

[1] Calculated from formula of von Pirquet.

[2] Reported by Mason and Benedict ('31).

PROCEDURE

For the measurements in Madras the subjects came to the laboratory on intermenstrual days at 7.00 A.M. and rested for at least half an hour before the beginning of the experiment. The usual hour for the preceding meal was the same as in the Indian series, 7.30 P.M. The point raised by Radsma ('31) that a half-hour rest period may not be long enough for tropical residents to achieve a state of repose has been discussed in a paper on the effect of sleep on metabolism (Mason and Benedict, '34). Mouth temperatures were taken during the rest period with the thermometer in position for at least 5 minutes. Reclining blood pressures were taken with a mercury manometer at the end of the experiment.

The apparatus used for measuring the oxygen consumption was the Benedict spirometer type, with graphic record of respiration. In all experiments through July, 1930, a mouthpiece was used; after that time the mouthpiece was replaced by the Benedict helmet (Benedict, '30).

The measurements in temperate climates were all made in well-established laboratories and clinics and the experimenters followed the customary procedures necessary to secure basal conditions.

RESULTS

A. Physiological measurements on European women in Madras and comparison with Indian women

In table 2 are shown the averages of measurements for each of the thirty-four women studied in Madras. All, except two, were measured on at least two consecutive or nearly consecutive days and those two had had trial experiments previous to the day recorded. The figures given represent, on an average, for pulse rate eighteen measurements, for respiration rate twelve measurements, blood pressure four measurements, and oxygen consumption six 10-minute periods of measurement. The recorded vital capacity was the highest of three readings. For comparison with these data, the corresponding data on Indian women reported in 1931 are included.

TABLE 2

Summary of measurements on European women in Madras

NUMBER	MOUTH TEMPERATURE °F.	PULSE RATE	BLOOD PRESSURE			RESPIRATION RATE	VITAL CAPACITY	O_2 CC./MIN.	HEAT PRODUCTION		PER CENT DEVIATION		
			Syst.	Diast.	P. Pr.				Per 24 hours	Per m²/hr.	Harris-Benedict	Dreyer	Aub-DuBois
									Calories	*Calories*			
1	98.8	57	108	62	46	11	2.80	182	1265	33.6	— 9.3	—10.5	—11.7
2	98.7	73	109	63	47	14	3.12	185	1286	33.9	— 7.6	— 8.1	— 8.4
3	97.9	64	97	60	38	14	4.24	166	1153	30.0	—16.1	—15.0	—18.9
4	98.4	49	94	47	47	16	4.05	194	1348	33.8	— 4.9	— 4.8	— 8.6
5	98.0	48	101	65	36	13	3.88	167	1160	30.2	—13.7	—10.6	—18.4
6	98.6	54	115	50	66	15	3.70	169	1171	31.5	—11.9	—10.2	—15.0
7	98.1	58	102	68	34	13	2.80	169	1171	34.6	— 6.8	— 4.9	— 6.5
8	98.7	58	115	57	58	12	2.93	156	1084	31.0	—14.4	—11.6	—16.4
9	97.9	61	104	69	36	15	2.63	172	1195	31.0	—14.0	—14.2	—16.4
10	98.7	71	118	76	42	11	3.72	176	1223	31.3	—10.3	— 8.3	—15.5
11	98.8	71	108	52	56	22	2.53	185	1283	34.2	— 4.4	— 3.3	— 7.6
12	99.0	63	127	77	50	15	4.17	173	1202	34.5	— 4.5	— 2.1	— 5.4
13	98.3	75	102	64	38	14	3.61	189	1313	32.4	— 8.3	— 8.2	—11.3
14	98.4	65	112	70	42	19	3.00	168	1164	31.9	— 9.6	— 7.3	—12.7
15	98.8	69	110	83	27	19	3.77	187	1299	32.6	— 5.3	— 3.5	—10.7
16	98.0	57	103	45	58	20	4.03	182	1265	33.5	— 2.9	+ 0.7	— 8.1
17	97.7	60	104	70	34	14	2.78	152	1056	28.6	—17.2	—13.3	—21.8
18	98.8	62	107	66	41	17	2.30	187	1299	32.6	— 3.6	— 0.4	—10.6
19	98.6	62	109	68	41	12	2.80	183	1272	34.6	— 0.8	+ 1.4	— 5.2
20	98.4	61	100	59	41	15	3.38	152	1056	29.0	—16.4	—14.2	—20.7
21	98.3	70	105	69	36	14	2.60	163	1130	32.9	— 5.6	— 2.4	— 9.9
22	98.9	49	105	44	62	16	3.30	176	1223	34.0	— 2.3	+ 0.2	— 6.9
23	98.7	72	98	61	37	21	2.83	163	1133	31.4	— 9.3	— 7.3	—13.8
24	98.4	63	106	74	32	11	3.55	198	1376	32.0	— 3.9	— 2.7	—12.2
25	97.6	74	109	68	41	15	3.60	175	1213	30.8	— 8.2	— 6.4	—14.4
26	99.0	80	121	79	42	13	3.17	187	1299	34.7	+ 2.4	+ 4.9	— 3.7
27	98.5	73	116	68	48	19	3.52	208	1442	33.2	— 0.7	+ 0.4	— 7.8
28	97.7	62	107	67	40	10	2.75	166	1150	30.9	— 9.2	— 7.1	—14.1
29	97.9	49	99	57	42	19	2.53	164	1136	31.6	— 5.8	— 3.3	—12.4
30	98.0	54	110	70	40	12	2.60	150	1042	28.8	— 15.7	—14.9	—20.1
31	98.0	65	105	62	43	17	2.43	174	1206	28.1	— 14.9	—14.4	—22.1
32	97.5	74	111	80	31	14	3.87	187	1296	32.7	+ 0.4	+ 1.3	— 9.2
33	98.6	67	109	71	38	15	3.05	161	1116	30.2	— 9.3	— 9.9	—13.8
34	98.0	63	128	81	47	8		170	1181	30.4	— 5.5	— 5.4	—13.2
Average 34 Europeans	98.3	63	108	65	43	15	3.21	175	1213	32.0	— 7.9	— 6.3	—12.5
Average 54 Indians	98.4	68	102	64	38	19	2.15[1]	150	1048	31.2	—16.9	—16.2	—17.2

[1] This figure is based on measurements of 853 south Indian women reported in 1932.

Mouth temperature. I have found in the literature very few data on early morning basal temperatures of women in temperate climates for comparison with this series in the tropics. The early morning mouth temperatures of thirteen young women studied by Gustafson and Benedict ('28) at Wellesley College averaged 97.7° F. Griffith and his collaborators ('29) found an average mouth temperature under basal conditions of 97.8° for three women studied over a long period of time, and the average early morning mouth temperature of five women reported by Harvey and Crockett ('32) was 97.7°. Brandt and Benjamin ('33 and unpublished data) found an average basal temperature of 98.0° for seven women and Turner (unpublished data) 98.0° for eighteen women students. The only exception to these low morning temperatures is a series of six women measured by Carpenter (unpublished data) during a hot spell in Boston in May and June. The average was 98.3°. The series in Madras averaged 98.3° for the Europeans and 98.4° for the Indians. The Madras temperatures early in the morning are therefore a little higher than those of women in temperate climates. It may be noted that eleven of the thirty-four European subjects, or 32 per cent, had temperatures higher than 98.6° even at 7.15 A.M. This was true also of thirteen, or 24 per cent, of the fifty-four south Indian women and I believe that these high temperatures should be recognized as within the normal range for women resident in the tropics.

Pulse rate. The average reclining pulse rate for the Europeans was 63 beats per minute. This is only half a beat less than the average for thirty-three American women in Benedict's second normal series ('28), but is 6 beats less than the earlier series of Harris and Benedict on 103 women ('19). These two series show approximately the same age range as the Madras series. In the series of twenty-five women 17 to 37 years of age reported by Turner ('27 a) the average reclining pulse was 65. These data therefore indicate that the reclining pulse rate of European women in Madras is as low or slightly lower than that of women in America. The higher

value for Indian women is probably due to their smaller body size rather than to a racial difference.

Blood pressure. Like body temperatures, data on reclining blood pressures of women in temperate climates are extremely scarce. The values found for women in Madras are within the range of reclining pressures indicated by Turner ('27 b) for young American women, but the systolic pressure of the Indian group is near the lower limit and in the European group, although twelve were 40 years or older, the systolic level is below the middle of Turner's range for young women. There is a suggestion, then, of a slightly lowered systolic pressure for women in Madras, but the difference may be found to be due to the fact that the group there were in the post-absorptive state. In view of the difference in the ages of the two groups, the small difference in systolic pressure between the Indian and European women is probably not significant.

Respiration rate. A comparison of the respiration rate with data obtained in a temperate climate is of questionable value because of possible varying effects on this measurement of the different breathing appliances used with metabolism apparatus. However, since the same apparatus was used with both groups of subjects in Madras, it is justifiable to conclude that there is a probable significance in the difference between the European respiration average of 15 per minute and the Indian average of 19. The more rapid rate in Indian women is probably a compensation for the very shallow breathing that characterizes their respiration records in comparison with the European records.

Vital capacity. The vital capacity of the European women is less than 3 per cent lower than the normal prediction of Turner ('30) for American women of the same height. There is, then, no evidence of a climatic effect on this function. The extraordinarily low vital capacity indicated here for the Indian women has been established in a series of measurements on 853 subjects reported in 1932.

Heat production. In comparing the heat production of European women in Madras with women in temperate climates and with Indian women, the difficulty is at once apparent that while the metabolism of Indian women is equally low whether compared with the Harris-Benedict, Dryer or Aub-DuBois standards, these same standards vary widely when applied to the European group. In fact, the difference between the deviations from the Dreyer and the Aub-DuBois standards in the European group is greater than the difference between the European and Indian groups by the Aub-DuBois standard. In the European group the deviations from the Aub-DuBois standards diverge more widely from the other two, particularly from the Harris-Benedict, with increasing age, and the interpretation of results is confused by this divergence. If the Harris-Benedict and Dryer standards are used, it may be concluded that the climatic effect on the metabolism of this group as a whole is small, especially if the 5 per cent reduction proposed by Benedict ('28) for women is applied, but between this group and the Indian group there is by the same two standards the significant difference of 9.0 and 10.2 per cent, which remains to be explained by factors other than climate. On the other hand, if the Aub-DuBois standards are applied, the climatic effect is much more marked and the difference between the two racial groups becomes less significant.

B. The effect of change of climate on European and Indian women

The confusion of interpretation that is presented by diverging standards is not present in a study of the same individuals as they move from one place to another. Such studies in which the effect of changes of climate on the same individuals has been measured are extremely few. They have been reported by Knipping ('23), Williams and Benedict ('28) and Martin ('30). Knipping measured fourteen European men during a voyage to the tropics. Unfortunately, none of the fourteen were measured on more than one day in their

home ports—a fact which detracts somewhat from the value of the tropical comparison. Following the original measurement, five were measured on 2 days in tropical ports (including Suez and the Red Sea) and nine on 1 day. The results show an average fall in oxygen consumption in the tropics of 2.3 per cent, with variations in different individuals of from + 8.0 to — 9.2 per cent. Although Knipping draws the conclusion that there is an initial rise in the metabolism on going to the tropics with return to normal and subsequent fall after prolonged stay, I think that the questions of the initial rise and the interval at which the fall occurs need further examination.

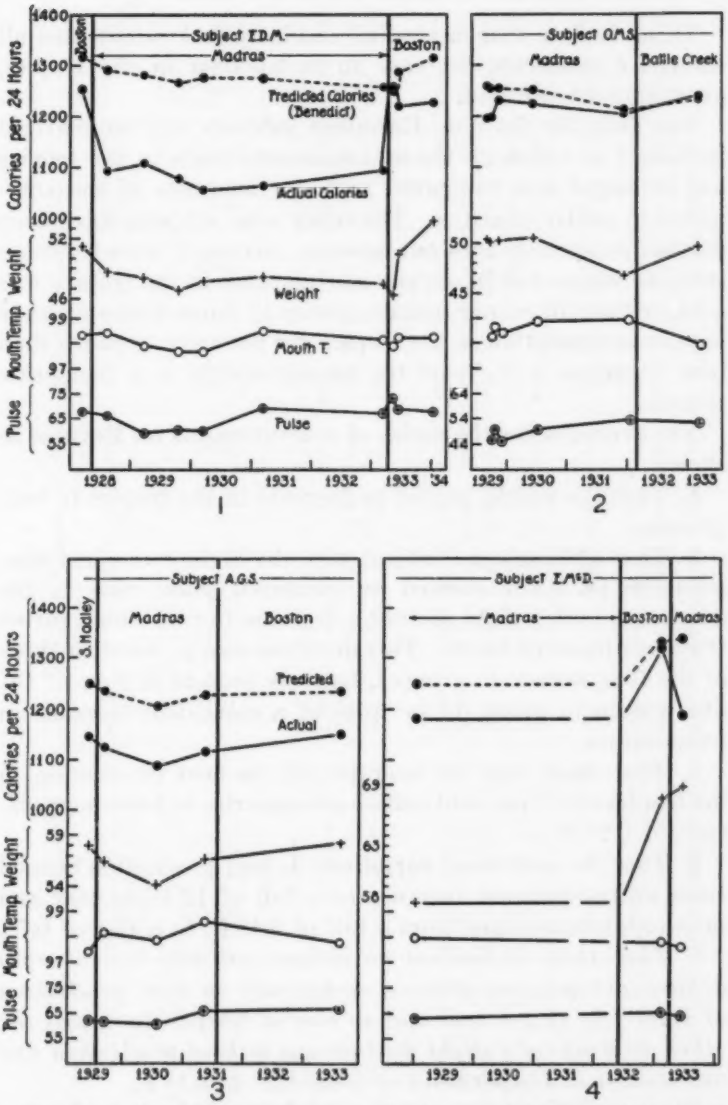
Williams and Benedict ('28) report four Americans measured in Boston and Yucatan. Here again the data are meagre, one preliminary measurement in Boston being followed by one or two in Yucatan and one after return to Boston. All four subjects showed a higher metabolism in Yucatan, varying from the insignificant rise of 1.0 per cent to 9.9 per cent and averaging + 6.4 per cent. It is significant that this change is in the same direction as the metabolism level reported by the same authors for Maya Indians in Yucatan, i.e., the metabolism of the white subjects was higher in Yucatan and the metabolism of the Maya Indians is high.

The most significant study of this kind, even though on only one individual, is that reported by Martin ('30). During a voyage from London to Australia he measured his own basal metabolism with the Douglas bag daily for the 35 days of the voyage, under very constant conditions as regards both food and exercise. Within a few days after encountering hot weather his metabolism began to fall, reaching a minimum on the fifth day of heat and remaining low until cooler weather was brought by the trade winds when it immediately rose to its previous level. The maximum difference between the hot and cool periods was 12 per cent. There is no question that in the case of this individual the tropical climate had a depressing effect on the basal metabolism.

Nine European women studied in Madras and in temperate climates did not show a uniform response to the tropical climate. Three showed a marked decrease in metabolism in the tropics from that in Boston, while a fourth showed a decrease that was definite, but less marked. The measurements on two of these subjects, E.D.M. and E.McD., who differed widely in age and bodily build, are charted in figures 1 and 4. In the most complete series, on E.D.M., the heat production had fallen to a level much below that found in Boston when it was measured for the first time in the tropics after 7 weeks' residence. It remained at this level throughout the 5-year period and when measured 4 days after landing in the U.S.A. had returned to the same level shown in the Boston measurements made 5 years earlier. That the tropical fall in metabolism in this subject was not due to the moderate loss in weight that had occurred in the course of a rough sea voyage is shown by the fact that the very marked rebound in metabolism on her return to a cold climate had taken place while the weight was still at its lowest level. The changes in heat production in the other three of these four subjects were also independent of changes in weight.

In contrast with these four subjects were four others who showed no significant changes in heat production as they moved from one climate to another and a fifth whose measurements showed considerable variation apparently not related to climate. The results for two of these second four, O.M.S. and A.G.S., are charted in figures 2 and 3. With A.G.S. the small fluctuations in heat production were in the same direction as changes in weight, but with O.M.S. the high level of heat production in the temperate climate was sustained in the tropics in spite of marked loss in weight.

In none of the subjects was there any indication that, apart from the effect of loss of weight, continued long stay in the tropics caused a progressive lowering of the metabolism, nor in the entire group of thirty-four women was there any relation between length of stay in the tropics and the level of heat production.



Figs. 1 to 4 The effect of change of climate on metabolism, weight, mouth temperature and pulse rate in European women. (Each point is the average of 2 days' measurements, except those on O.M.S. in Battle Creek which are averaged from 4 days.)

Three Indian women studied similarly in two climates all showed a moderate decrease in metabolism in the tropics, averaging 4.8 per cent.

The data for the nine European subjects are summarized in table 3 in which all the measurements made in the tropics are averaged and compared with the averages of measurements in colder climates. The other nine subjects have been divided arbitrarily into two groups. Group I includes those subjects whose fall in oxygen consumption in the tropics was greater than 10 cc. per minute, group II those whose average oxygen consumption in the tropics did not vary by more than plus or minus 5 cc. from the measurements in a temperate climate.

The averages for the series of measurements on Europeans show:

1. That the weight tended to decrease in the tropics in both groups.

2. That although the subject with the highest tropical temperature (E.M.C.) showed an increased pulse rate in the tropics, the other eight showed a decrease in rate which varied from one to seven beats. This decrease was greatest in three of the four women in group I, but was present in four of the five women in group II in spite of a coincident increase in temperature.

3. That there was an average fall in heat production in the tropics of 5.1 per cent and an average rise in body temperature of 0.2° F.

4. That the individual variations in heat production ranged from an insignificant increase to a fall of 12.4 per cent and in mouth temperature from a fall of 0.4° F. to a rise of 0.7°.

5. *That these individual variations fell into two sharply defined groups, one showing a decrease in heat production of from 7 to 12 per cent and no rise in temperature, and the other showing only slight fluctuations in heat production and an increase in temperature of from 0.2° to 0.7° F.*

The correlation between per cent change in heat production and change of temperature in the tropics was found to be

TABLE 3

Summary of experiments on effect of change of climate on European women

SUBJECT	HEIGHT	AGE AT START	METABOLISM PER CENT DEVIATION IN TEMPERATE CLIMATE		WEIGHT, KILOGRAM			MOUTH TEMPERATURE °F.			PULSE RATE			O_2 CONSUMPTION CC. PER MIN.		HEAT PRODUCTION CALORIES PER 24 HOURS		
			Harris-Benedict	Aub-DuBois	Temp.	Trop.	Per cent change	Temp.	Trop.	Change	Temp.	Trop.	Per cent change	Temp.	Trop.	Temp.	Trop.	Per cent change
Group I	*cm.*																	
E.L.C.	154	25	— 4.0	— 2.9	44.0	44.8	+1.8	98.2	98.2	±0	64	58	—9.4	172	160	1195	1112	— 6.9
E.D.M.	169	30	— 4.0	—10.1	50.4	47.7	—5.4	98.1	98.1	±0	70	64	—8.6	178	156	1237	1084	—12.4
G.E.C.	167	44	— 4.6	—12.0	72.0	70.0	—2.8	98.5	98.1	—0.4	72	65	—9.7	199	176	1383	1223	—11.6
E.McD.	164	55	— 0.6	— 9.6	67.6	63.1	—6.7	97.8	97.8	±0	66	64	—3.0	190	171	1320	1188	—10.0
Group II																		
B.S.C.	157	26	—15.4	—16.8	61.4	58.8	—4.2	98.0	98.2	+0.2	62	61	—1.6	171	169	1188	1174	— 1.2
K.N.B.	167	37	—11.6	—17.6	58.0	58.4	+0.7	97.6	97.9	+0.3	77	75	—2.6	170	172	1181	1195	+ 1.2
O.M.S.	159	37	+ 0.2	— 3.9	49.6	49.5	—0.2	97.9	98.6	+0.7	52	49	—5.8	178	174	1237	1209	— 2.3
E.M.C.	165	40	— 1.8	— 7.8	56.6	53.9	—4.8	98.5	98.8	+0.3	74	85	(+14.9)[1]	186	187	1292	1299	+ 0.5
A.G.S.	154	52	— 7.2	—11.7	58.1	55.7	—4.1	97.6	98.2	+0.6	66	63	—4.5	165	160	1146	1112	— 3.0
Average All subjects			— 5.4	—10.3	57.5	55.8	—3.0	98.0	98.2	+0.2	67	65	—5.0[1]	179	169	1242	1177	— 5.1
Group I			— 3.3	— 8.7	58.5	56.4	—3.3	98.2	98.05	—0.15	68	63	—7.7	185	166	1284	1152	—10.2
Group II			— 7.2	—11.6	56.7	55.3	—2.5	97.9	98.3	+0.4	66	67	—3.6[1]	174	172	1209	1198	— 1.0

[1] Including E.M.C. the average change in pulse rate for all subjects is —3.4 per cent and for group II +0.1 per cent.

0.717 ± 0.109, that between change of metabolism and change of pulse 0.627 ± 0.136. A very interesting physiological adaptation to the tropics is suggested by the first high correlation, namely, that those individuals who are able to diminish their heat production in the tropics are thereby able to avoid a rise of temperature. They may even overdo the adaptation, as is suggested by the temperature curve of E.D.M. (fig. 1), and lower their heat production to such an extent that the body temperature also is lowered. On the other hand, in those individuals who do not respond to the tropical climate with a decrease in heat production, the mechanism for heat loss is not adequate to prevent a rise in temperature.

Apart from this relation between change in metabolism and change in temperature and to a less extent between change in metabolism and change in pulse, there was no uniformity within the two groups, the individuals in each group showing a scattering with respect to race, configuration and previous association with the tropics (table 1) as well as to the initial level in temperate climates of metabolism, temperature and pulse.

The highly suggestive correlation discussed above between change in temperature and change in metabolism is based on only nine fairly scattered points and therefore can be considered as only indicative unless confirmed by further data. However, fairly strong confirmation is provided by an analysis of the relations between individuals in the larger group of thirty-four women measured in the tropics. In this group there was the significant correlation of 0.530 ± 0.083 between mouth temperature and heat production per square meter—a fact which gives considerable support to the adaptation suggested by the group of nine. On the other hand, the less strongly indicated correlation between change in pulse rate and change in metabolism is not supported by the larger group who showed no significant correlation between pulse and metabolism.

Until a long continued study of many individuals has been made, it is possible only to speculate as to whether individuals

showing both types of response to the tropics indicated above may exist in large numbers, or whether an individual who at one time responds in one way may at another time respond differently. It is probable that as the study is continued the division will be found to be less sharp. The fact that the average temperature of the group of thirty-four women in Madras is as high as that of the women in group II suggests that individuals of the second type, whose metabolism remains unchanged, but whose temperature is raised, may predominate in this group.

FACTORS OTHER THAN CLIMATE

In discussions of white races living in the tropics one encounters repeatedly the impression that such tropical residents lead a rather indolent existence and that this lack of physical activity accounts for any physiological changes that may occur. In the case of the women studied in Madras this traditional inactivity is certainly a fiction. Of the thirty-four women whose measurements are reported here, thirty were educational or medical missionaries leading exceedingly busy lives and the remaining four were also engaged in exacting activities. In a study of a group composed largely of missionaries one would be inclined to suspect fatigue rather than inactivity as a factor likely to affect metabolism. That this was not a factor is shown from a study of the data presented with relation to the time since last arrival in India. There is no correlation between the time of residence in India and the metabolism either in the group as a whole or in individuals studied through progressive stay in Madras.

The same differences in recreational activity that would be found in a similar group in a temperate environment were apparent here. Individuals of athletic habit at home continue the athletic habit in India, in the forms of tennis, swimming, bicycling, riding, walking, and, in the holidays, climbing.

With regard to diet also, there is a much less radical change than is frequently supposed. While many Europeans have rice and curry frequently, their diet is predominantly Euro-

pean and, as far as I have observed, does not show any indication of decrease in quantity. At the Women's Christian College, where sixteen of the subjects were resident, breakfast, tiffin and dinner were as hearty as the three corresponding meals of the same class of women in America and, in addition, there was a light early morning choti (tea, toast and fruit) and a substantial tea.

While it does not seem probable that there are changes in activity and diet sufficient to account for the changes in metabolism that occur in some individuals, these factors cannot be dismissed with certainty until they have been carefully investigated.

With regard to the influence of factors other than climate in the case of the three Indian subjects measured in temperate climates, it may be stated that all three were engaged in scientific work both in Madras and abroad, that one, a keen botanist, resumed her botanical expeditions on her return to Madras and played tennis. During her first year in Ann Arbor she had lived on a predominantly American diet, but during her second year there returned entirely to her normal Indian vegetarian diet. The second, an excellent tennis player, continued her vigorous game when she returned from Toronto to Madras, and by preference lived on the same diet as her European colleagues. In the case of these two, then, of their own accord the factors of diet and activity were controlled and only climate varied. The third subject was a physically inactive person in Madras and was not given to exercise in London where she was living in a student hostel.

CONCLUSION

Returning from the study of these individuals undergoing change of climate to the question of the importance of the climatic factor in the average metabolism level of south Indian women, the following tentative conclusions may be drawn: that since the small group of three Indians showed an average rise of metabolism in colder climates of 4.8 per cent and the group of nine Europeans showed an average decrease in the

tropics of 5.1 per cent, probably at least 5 per cent of the low metabolic level of Indian women may be attributed to the effect of tropical climate. If the 5 per cent correction of standards for women be applied in addition to the correction for climate there still remains to be explained by factors other than climate a difference between the average metabolism of Indian women and western women of approximately 7 per cent.

SUMMARY

Measurements on thirty-four European women resident in the city of Madras, which has a mean annual temperature of 82.8° F. and a relative humidity of 72 per cent, showed an average metabolism 7.9, 6.3 and 12.5 per cent below the Harris-Benedict, Dreyer and Aub-DuBois standards, respectively. By the same standards this was 9.0, 9.9 and 4.7 per cent above the average metabolism of Indian women in Madras. The mouth temperature was slightly higher than reported in Western countries and the pulse and blood pressure as low or slightly lower. These three measurements do not differ significantly from those on Indians. The European vital capacity was normal and very much higher than that of Indian women.

Nine European women studied in both temperate and tropical climates showed two types of response to the tropics. One group showed a marked decrease in metabolism, a fairly marked fall in pulse rate and no rise in mouth temperature. The other group showed no change in metabolism, a slight fall in pulse rate and a rise in temperature of from 0.2° F. to 0.7°. The high correlation found in this small group of subjects between change in metabolism and change in temperature is supported by a high correlation between temperature and metabolism in the series of thirty-four women measured in Madras. The average decrease in metabolism of the group of nine moving to the tropics was 5.1 per cent. Three Indian women measured in two climates showed an increase in metabolism of 4.8 per cent in cold climates. These data sug-

gest that approximately 5 per cent of the low metabolism previously reported for Indian women may be attributed to the effect of tropical climate.

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THE ASSIMILATION OF PHOSPHORUS FROM DICALCIUM PHOSPHATE, C.P., TRICALCIUM PHOSPHATE, C.P., BONE DICALCIUM PHOSPHATE AND COOKED BONEMEAL

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In a review, briefly published ('32) by one of the writers of experiments dealing with the relative value of different forms of calcium and phosphorus as mineral supplements for bone formation, the preponderance of the evidence failed to show any differences among the various forms. However, there were certain possible upsetting factors present in most of these experiments which suggested that the negative data should not be accepted as final. In very few of the comparisons could it be said with certainty that the level of intake was sufficiently low to bring out any difference in efficiency which might exist. In many of the studies involving sources of both calcium and phosphorus, only one of these elements was held constant, with a resulting variable calcium-phosphorus ratio. It seemed worth while therefore, to study this problem further, with special attention to the elimination of these variable factors. The present paper describes such studies with various calcium phosphates.

In planning these studies it became apparent that it is impossible to make a critical comparison of the assimilation of both calcium and phosphorus in the same experiment. For example, if dicalcium phosphate and tricalcium phosphate are introduced into diets 1 and 2, respectively, at the same calcium level, diet 1 will have the more phosphorus and the diets will

differ as regards calcium-phosphorus ratio. This is not a satisfactory situation for the study of the assimilation of either element. The phosphorus levels can be equalized by adding phosphate to diet 2. This provides a suitable set-up for comparing the calcium phosphates as regards calcium assimilation, but not phosphorus, since another source of the latter has been added. Similarly, a satisfactory comparison of the phosphorus of the two sources can be provided for by introducing them at the same phosphorus level and adding more calcium to the dicalcium-phosphate diet. Thus it is possible to study only one of the elements at a time. The

TABLE 1

Analyses of mineral supplements

	CALCIUM	PHOSPHORUS
	per cent	*per cent*
Dicalcium phosphate, c.p.	24.36	20.49
Tricalcium phosphate, c.p.	36.80	19.00
Bone dicalcium phosphate[1]	23.28	18.67
Cooked bonemeal[1]	31.50	14.84

[1] Obtained from United Chemical and Organic Products Company, Chicago.

experiments here reported deal with the assimilation of phosphorus from the supplements shown in table 1. The bone dicalcium phosphate and the cooked bonemeal were products prepared for human use, being by-products of the manufacture of edible gelatin. Three separate experiments have been carried out.

Comparison of dicalcium phosphate, c.p., and cooked bonemeal

For this comparison a basal diet low in both calcium and phosphorus was selected as follows:

Butter	10
Patent flour	80
Lactalbumin	7
Yeast concentrate, Harris	1
Salt mixture	2

The salt mixture was a modification of the Osborne and Mendel product, free from calcium and phosphorus. This basal diet contained 0.05 per cent of calcium and 0.14 per cent of phosphorus. Realizing the importance of making the comparison with the element under study at a minimum level, as has been mentioned, and being uncertain as to what that level should be, it was decided to use three different levels in this first comparison. It was decided to maintain a calcium-phosphorus ratio of approximately 1.5 to 1 at each level by the addition of the required amount of calcium carbonate. The first six diets shown in table 2 were accordingly made up by combining appropriate amounts of the supplements with the basal diet. It is seen from the analytical data in the last two lines of the table that diets 1 and 2 provided a comparison at a level of 0.25 per cent, diets 3 and 4 at a level of 0.36 per cent and diets 5 and 6 at a level of 0.47 per cent, the calcium level being approximately 50 per cent higher in each case.

Six pairs of rats, weighing approximately 35 gm. at 23 days of age were selected for each level and fed for 35 days, limiting the food consumption of each pair to the intake of the one consuming the least. At the close of the feeding period the bones of the left hind leg of each rat were excised and analyzed for ash on a dry fat-free basis.

TABLE 2

Diets used in growth experiments

INGREDIENTS	DIETS									
	1	2	3	4	5	6	7	8	9	Control
Basal diet	100	100	100	100	100	100	100	100	100	100
Dicalcium phosphate, e.p.	0.48		1.01		1.52			0.54		
Bonemeal		0.30		1.28		1.97				
Bone dicalcium phosphate							0.59			
Tricalcium phosphate									0.58	
Calcium carbonate	0.58	0.41	0.75	0.31	0.83	0.20	0.58	0.58	0.47	0.53
Calcium, per cent	0.38	0.38	0.55	0.55	0.70	0.70	0.38	0.39	0.38	0.21
Phosphorus, per cent	0.25	0.25	0.36	0.36	0.47	0.47	0.28	0.29	0.30	0.17

Results. The bone data from the comparisons at the three phosphorus levels are presented in table 3. At the 0.25 per cent level the figures for percentage of ash favor the dicalcium phosphate in the case of five of the six pairs but, since the odds are only 2 to 1 according to Students' method, the differences have no significance statistically. The weights of bone ash, obtained by multiplying the weight of the dry, fat-free bone by its ash percentage favor the dicalcium phosphate in five out of six of the cases, with odds of 58.5 to 1. At the 0.36 per cent level the data for ash percentage favor the bonemeal in five out of six of the comparisons, but with odds of only 8.6 to 1. The figures for amount of ash favor one supplement in three cases and the other supplement in the other three. At the upper phosphorus level the percentage figures are in favor of the dicalcium phosphate in the case of five out of six of the pairs, with odds of 50.8 to 1. The supplement is also favored as regards bone ash in all cases, with odds of 39 to 1. In summary, the data for percentage ash favor dicalcium phosphate at the lower and upper levels, and the bonemeal at the intermediate level, while the data for amount of ash favor the dicalcium phosphate at all levels. The lack of consistency for the percentage data and the low odds shown for most of the differences rule out any definite conclusion in favor of dicalcium phosphate, from the experiment as a whole.

It is evident from the data that for both supplements the ash percentages were markedly lower at the level of 0.25 per cent phosphorus than at the other two. The average for all the animals fed at the lower level is 50.57 per cent, for the intermediate level, 53.98 per cent and for the upper level, 54.48 per cent. Thus it is clear that at least the level of 0.25 per cent was below that of maximum possible assimilation and that any marked differences in efficiency between the two supplements should have become evident at this level.

TABLE 3

Comparison of phosphorus assimilation from dicalcium phosphate, C.P. and cooked bonemeal at different phosphorus levels

	PHOSPHORUS LEVEL	PAIR 1		PAIR 2		PAIR 3		PAIR 4		PAIR 5		PAIR 6	
		Dicalcium phosphate, c.p.	Bone meal	Dicalcium phosphate, c.p.	Bone meal	Dicalcium phosphate, c.p.	Bone meal	Dicalcium phosphate, c.p.	Bone meal	Dicalcium phosphate, c.p.	Bone meal	Dicalcium phosphate, c.p.	Bone meal
	per cent												
Ash in dry, fat-free bone, per cent	0.25	48.44	47.43	51.63	50.64	49.77	48.91	50.81	54.23	51.94	48.75	54.13	50.16
Ash in dry, fat-free bone, mg.	0.25	191.0	157.7	190.3	154.7	179.2	166.3	152.3	171.3	161.6	136.1	141.0	124.0
Ash in dry, fat-free bone, per cent	0.36	53.43	55.86	51.44	54.07	53.66	53.82	54.41	55.54	55.26	53.92	52.99	53.33
Ash in dry, fat-free bone, mg.	0.36	212.0	226.7	198.7	205.2	212.0	218.9	189.3	165.9	203.7	182.4	169.1	139.2
Ash in dry, fat-free bone, per cent	0.47	54.34	54.37	54.45	52.01	52.87	52.36	55.77	54.78	56.44	55.87	56.76	54.84
Ash in dry, fat-free bone, mg.	0.47	250.5	227.0	177.5	176.2	198.0	176.7	196.3	192.0	214.6	212.6	246.5	212.0

Comparison of dicalcium phosphate, c.p., bone dicalcium phosphate and tricalcium phosphate, c.p.

In view of the data obtained in the first comparison, it was decided to conduct further ones at a phosphorus level of 0.25 per cent, as representing one which was certainly below that of maximum possible phosphorus storage. Diets 7, 8 and 9 listed in table 2 were accordingly made up for use in a second experiment. As is shown in the last line of the table, when the diets were analyzed they were found to be somewhat higher in phosphorus than the planned level. This was due to a larger amount in a new supply of lactalbumin obtained for the basal diet. This larger amount also resulted in a calcium-phosphorus ratio of approximately 1.3 to 1 instead of the 1.5 to 1 used in the first experiment. For comparative purposes, it was desired to ascertain the kind of bone which would be produced at the phosphorus level supplied by the basal ration alone. Thus enough calcium was added to it to provide the same ratio between the elements which was present in the experimental diets. This combination is listed as the control diet in the last column of table 2.

Since three experimental diets were involved, the rats were selected by trios instead of by pairs. Eight trios were used and for a given trio the food intake of the rat consuming the least determined the allowance for all. In addition to these rats distributed among the experimental diets, four comparable animals were placed on the control diet and fed an amount corresponding to the average intake of the other diets. In addition, four comparable animals were killed at the start of the experiment to obtain a measure of the ash content of the bones at this time.

Results. The data obtained in this experiment are shown in table 4. In discussing them it is preferable to consider the figures for two supplements at a time. As regards the comparison of dicalcium phosphate, c.p. with tricalcium phosphate, c.p. the data for percentage ash favor the secondary product in five out of the eight cases, but the odds are only 1.5 to 1. A clearer picture is presented by the figures for amount of

TABLE 4

Comparison of phosphorus assimilation from dicalcium phosphate, C.P., bone dicalcium phosphate and tricalcium phosphate, C.P.

	DICALCIUM PHOSPHATE C.P.	TRICALCIUM PHOSPHATE, C.P.	BONE DICALCIUM PHOSPHATE	DICALCIUM PHOSPHATE C.P.	TRICALCIUM PHOSPHATE, C.P.	BONE DICALCIUM PHOSPHATE	DICALCIUM PHOSPHATE C.P.	TRICALCIUM PHOSPHATE, C.P.	BONE DICALCIUM PHOSPHATE	DICALCIUM PHOSPHATE C.P.	TRICALCIUM PHOSPHATE, C.P.	BONE DICALCIUM PHOSPHATE
	Trio 1			Trio 2			Trio 3			Trio 4		
Ash in dry, fat-free bone, per cent	53.46	54.99	54.28	53.68	50.15	52.09	51.10	53.34	53.46	51.58	55.79	55.06
Ash in dry, fat-free bone, mg.	277.5	235.1	256.9	209.1	199.2	223.7	195.9	205.0	200.1	261.9	236.9	277.3
	Trio 5			Trio 6			Trio 7			Trio 8		
Ash in dry, fat-free bone, per cent	53.15	53.91	54.83	57.82	55.35	57.29	56.37	54.73	57.72	55.78	53.43	55.34
Ash in dry, fat-free bone, mg.	227.3	226.8	227.2	236.0	220.9	268.5	231.1	209.9	234.3	227.0	205.5	242.6

ash, since they favor the secondary product in all but one comparison, with odds of 70 to 1. Comparing the data from the bone dicalcium phosphate with those from the tricalcium phosphate, it is found that the bone product resulted in a higher ash percentage in six out of eight cases, with odds of 40 to 1. Similarly, the figures for amounts of ash are larger for the bone product in seven out of the eight comparisons, the odds being 26 to 1. These results which generally tend to favor the dicalcium phosphate, c.p. and the bone product over the tricalcium phosphate were obtained despite the slightly higher percentage of phosphorus in the diet containing the tertiary phosphate, shown in table 2. As regards a comparison of the data for dicalcium phosphate, c.p. and the bone product the percentage data favor the latter in five out of eight cases, but with odds of only 9.4 to 1. The differences in ash content are in the same direction in six out of eight comparisons, but again the odds are very small. Thus none of the data significantly favor one dicalcium phosphate over the other.

Summarizing the data in table 4, no evidence is presented for any difference between the two dicalcium phosphates. However, both of them produced, on the average, higher ash percentages and larger amounts of ash than resulted with the tricalcium phosphate. But the differences are rather small and not accompanied by adequate odds in all cases. Thus, while the data suggest a more efficient utilization of the phosphous in dicalcium phosphate than in the tertiary product, in agreement with the majority of the data in the first experiment; it is unsafe to consider this entirely proved. Certainly, no large differences exist, and in practice where it may be expected that any supplement used would be added in amounts above the minimum level any small difference in efficiency would become of no importance.

It is evident from the data in table 2 that the supplements furnished only about one-half of the phosphorus intake, and that the results measure the combined effect of the minerals in the basal diet and in the supplements. However, the data

obtained for the rats fed the control diet showed that it produced very poor bone by itself. The bones of the check animals killed at the start contained, on the average, 43.4 per cent of ash. The animals carried through on the control diet, while they made approximately 85 per cent of the gains made where the supplements were used, developed a very poor bone. The bones averaged only 44.1 per cent of ash, compared to an average value of over 54 per cent for the bones of the rats fed the supplements. When these figures are compared with the value for the check animals killed at the start the

TABLE 5

Diets used in growth and lactation experiment

INGREDIENT	DIETS		
	Control (10)	Bone dicalcium phosphate (11)	Bone meal (12)
Butter	10	10	10
Patent flour	80	80	80
Yeast concentrate, Harris	1	1	1
Salt mixture	2	2	2
Lactalbumin	7	7	7
Bone dicalcium phosphate		0.51	
Cooked bonemeal			0.61
Calcium carbonate	0.53	0.65	0.74
Calcium, per cent	0.25	0.43	0.43
Phosphorus, per cent	0.17	0.28	0.28

large influence of the added supplements is evident. Clear proof is furnished that the phosphorus of all the supplements was utilized for bone development.

A comparison of bone dicalcium phosphate and cooked bonemeal for growth and lactation

In this experiment the supplements were studied with female rats carried from weaning through their first lactation. The diets used are shown in table 5. It is noted that the control diet and the phosphorus levels were the same as in the second experiment. Ten rats were placed on each diet

at weaning and fed ad libitum. At 4 months of age they were mated with males of proved fertility and at parturition the young of each litter were reduced to six. Eighteen days later, mother and young were killed, after taking a blood sample from the heart of the mother for the determination of inorganic phosphorus. The left femur of the mother was removed for ash analysis. The object of this procedure was to measure the value of the supplements, not only in terms of growth of mother and young, but also as to the state of the phosphorus nutrition of the mother at the close of the lactation period.

All of the animals placed on the experiment lived, grew at a nearly normal rate and were normal in appearance when bred at 4 months of age. As was anticipated, a few of them failed to produce young. Any animal which failed to become pregnant within 2 months after being placed with the male was killed, since it was considered that even though such an animal should prove fertile later the results would be of little value for consideration along with the others of the group. Since only four of the thirty rats were thus killed without producing young, with a distribution of two in one group and one each in the others, as is seen in table 6, this factor does not seriously complicate the interpretation of the results.

Results. The data obtained are summarized in table 6. Two sets of averages are presented for each group, one for all of the animals and one for those producing young. The latter is clearly the more useful measure in this study. The gains in weight cover the period from weaning to the time the animals were mated, since after that time the changes in weight were influenced by the onset of pregnancy. These data for gains reveal very good growth in all groups, nearly as good as the average for our stock females. The largest increases were made by the group receiving the dicalcium phosphate. Though suggestive, the differences between the average gains for this group and the other two are not statistically significant. Also it is recognized that without food intake records the possibility of differences in growth

TABLE 6

Comparison of bone dicalcium phosphate and cooked bonemeal for growth and lactation

DIET	RAT NO.	GAIN FROM WEANING TO 4 MONTHS	ASH WHEN KILLED	NUMBER OF YOUNG BORN	WEIGHT OF YOUNG REARED	WEIGHT DRY FAT-FREE FEMUR	PER CENT ASH IN DRY FAT-FREE FEMUR	INORGANIC P IN PLASMA
		gm.	*days*		*gm.*	*gm.*		*mg./100*
Control (10)	1	160	206	0	0	0.3813	64.67	5.7
	2	111	160	6	109	0.2267	54.92	1.4
	3	139	206	0	0	0.3567	66.41	4.9
	4	179	188	7	65	0.3378	61.87	6.0
	5	177	169	5	91	0.2714	57.36	5.8
	6	151	166	6	132	0.2796	58.96	0.6
	17	159	166	4	118	0.2933	56.97	2.8
	18	146	165	6	114	0.2274	56.49	0.7
	19	151	163	9	20	0.3314	61.59	5.4
	20	200	167	7	114	0.2234	57.21	1.8
Average for group		157	176	5.0	76	0.2929	59.64	3.51
Average for fertile rats (8)		159	168	6.2	95	0.2739	58.17	3.06
Bone dicalcium phosphate	7	157	168	5	142	0.3310	62.60	5.0
	8	181	205	7	106	0.3916	64.58	6.5
	9	151	161	6	138	0.3483	63.31	2.8
	10	179	201	8	142	0.3661	64.00	5.0
	11	204	174	7	156	0.3262	63.18	5.7
	26	147	169	6	60	0.4016	65.88	5.3
	27	194	160	4	65	0.4572	67.86	6.3
	28	228	214	0	0	0.4440	66.05	5.5
	29	151	164	6	76	0.3388	64.99	8.5
	30	182	201	8	150	0.3677	62.17	2.5
Average for group		177	182	5.7	103	0.3772	64.46	5.31
Average for fertile rats (9)		172	178	6.3	115	0.3698	64.30	5.29
Cooked bonemeal (12)	12	153	161	5	125	0.3336	62.32	1.9
	13	163	166	5	101	0.3461	63.85	5.2
	14	158	165	4	104	0.3255	63.32	5.6
	15	164	167	6	124	0.2783	63.24	2.6
	16	169	162	6	130	0.3212	62.30	5.0
	21	138	176	6	45	0.3570	64.43	5.0
	22	152	210	0	0	0.4215	65.35	5.0
	23	126	165	5	123	0.3142	64.86	5.5
	24	170	172	7	178	0.3418	63.96	4.4
	25	174	168	9	68	0.4191	66.52	7.0
Average for group		157	171	5.3	100	0.3458	64.01	4.73
Average for fertile rats (9)		157	167	5.9	111	0.3374	63.87	4.70

being due primarily to differences in food intake cannot be ruled out. The striking feature of these growth data is that the very low level of phosphorus in the control diet did not prevent a nearly normal increase in weight. As a result the three groups were nearly alike in weight, as well as being of the same age, when subjected to the test of reproduction and lactation. The data for age when killed are pertinent in connection with the figures for weight and ash content of femur, since the latter values increase with age. It is noted that the group receiving the dicalcium phosphate had a slight advantage in this respect. This was due to the variations in the time elapsing before pregnancy ensued following mating.

The data for reproduction show that two females on the control diet failed to bear young in the time allotted and that the same was true for one animal in each of the other groups. As a whole this represents very good fertility in terms of the records of our colony. For those producing young the average number born is slightly lower for the bonemeal group than for the other two. These averages, as a whole, are close to what may be expected for first litters in our colony. Thus the data cannot be considered to present any evidence that the low phosphorus intake of the control diet interfered with reproduction or that any group was superior to another in this respect.

The weight of young reared constitutes a measure of lactation performance. Since a few of the mothers failed to produce as many as six young, the planned reduction of the litters to this number did not result in entire equality for the lactation study. The differences here were not large, as shown by the following data for the fertile animals:

GROUP	AVERAGE NUMBER AT START OF LACTATION	AVERAGE NUMBER AT END OF LACTATION
Control	5.5	4.7
Dicalcium phosphate	5.7	4.6
Bonemeal	5.3	4.7

These data also indicate the losses which occurred in the course of the lactation and provide a basis for calculating the average individual weight at the close. These lactation data show large individual variations within the groups. For example, in the basal group rat 6 reared all of her six young to a total weight of 132 gm., while rat 19, which gave birth to nine young, reared only one out of six, to a weight of 20 gm. While the highly variable individual data keep the differences from being statistically significant, it is noted that the average values for weight of young reared are distinctly higher for the groups receiving the supplements than for the control, suggesting a beneficial effect from the phosphorus additions.

The more critical measures, however, are the bone data. In this connection it is noted in the table that the average age of the animals when killed was substantially the same for the control and the bonemeal groups, and slightly higher for the other group. The same relations were noted for the weights of the animals when killed. Thus, although the phosphorus additions did not have any marked effect on the growth in weight of the mothers, it is clear from the data for weight of femur that they did markedly influence the growth of bone.

A comparison of the data for diets 10 and 12, for which the average values for weight and age of mothers were nearly identical, shows an increase in femur weight of approximately 23 per cent as a result of the bonemeal addition. The difference is 4.6 times its probable error. For the group on diet 11, in which the average weight of the animals when killed was approximately 15 per cent greater than for the control group, the average femur weight is seen to be 35 per cent higher, the difference being 6.6 times its probable error. While the average value for femur weight is higher for the dicalcium phosphate group than for the group receiving bonemeal, the difference is less than three times its probable error. Further, a higher value is to be expected, since the animals receiving the dicalcium phosphate were heavier and older when killed. Thus no reliable evidence in its favor is here furnished.

The data for ash percentage show clearly that the supplements resulted in a bone of better quality, as well as the larger growth previously discussed. The difference between the average values for the control group and for the dicalcium phosphate group is over eight times its probable error. The difference favoring the bonemeal group over the control has an even higher significance attached to it. It is noted that the values for the two groups receiving the supplements are substantially alike. Experiments with various species have shown that during lactation the calcium and phosphorus in the milk may be in part supplied by a withdrawal of these elements from the bones. It is of especial significance, therefore, that although the groups receiving the supplements presumably secreted more milk and thus more of the minerals, as indicated by the greater weights of young weaned, their bones remained markedly higher in ash at the end.

The data for inorganic phosphorus in the plasma, while highly variable, serve to confirm the conclusions thus far drawn as to the better state of mineral nutrition caused by the addition of the supplements. The difference between the values for the two supplements has no statistical significance.

The foregoing discussion of the bone and blood data in table 6 has been made on the basis of the average values for the animals rearing young, since they represent the more critical measures. It is interesting to compare the data for the rats not producing young in the control group with the average value for the others of the group. For these two rats the figures for weight of bone, ash content and blood phosphorus are all higher than the corresponding average values for the others. This would seem to reveal the draft on the bones which especially results when an animal in a poor state of calcium and phosphorus nutrition is subjected to the strain of lactation. No such differences are shown in the other two groups, but comparison here is of little value, because only one animal in each group did not lactate.

As a whole, the data in table 6 show that, while the low mineral content of the basal diet did not interfere noticeably with growth in weight and thus that no superiority for either supplement was revealed in this respect, their addition did markedly improve bone development. Better bones was undoubtedly produced during the growing period, and this bone presumably remained more nearly intact during pregnancy and lactation than did the bone of the animals on the mineral-deficient, basal diet. At any rate, the animals receiving the supplements were in a much better state of calcium and phosphorus nutrition at the close of the lactation. The usefulness of both bonemeal and dicalcium phosphate as supplements to a mineral--deficient diet is thus clearly shown.

SUMMARY AND CONCLUSIONS

Three experiments have been carried out with rats in which the phosphorus intake was kept at a minimum level, and in which the calcium-phosphorus ratio was held constant in the diets under comparison. In the first experiment dicalcium phosphate, c.p., and cooked bonemeal were compared by the paired feeding method at three levels of phosphorus intake, using growth and bone development as the criteria. In the second experiment a similar procedure was used to compare dicalcium phosphate, c.p., bone dicalcium phosphate and tricalcium phosphate, c.p. In the third experiment bone dicalcium phosphate and cooked bonemeal were studied with female rats carried from weaning through their first lactation. In all of these experiments the usefulness of the supplements was shown by the data for ash content of the bones. In the last experiment, although the rats receiving the supplements reared a greater weight of young, their bones remained much higher in both percentage and amount of ash than did those of a control group receiving no supplement. This evidence of a better state of calcium and phosphorus nutrition was supported by data showing a higher level of inorganic phosphorus in the blood. Throughout these three experiments such differences as were shown between the supplements

under comparison generally favored a secondary phosphate over a tertiary product, but we are unwilling to consider this combined evidence as certain proof, because of the small differences involved and the variability of the individual data. It seems very unlikely that any differences which may actually exist are large enough to be of appreciable importance in the selection and use of mineral supplements in practice.

LITERATURE CITED

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THE SEASONAL VARIATION IN THE ANTIRACHITIC EFFECTIVENESS OF SUNSHINE [1,2]

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The fact that sunshine has distinct antirachitic properties has been so definitely established that citation of further proof is no longer necessary. The results of numerous investigators, however, show that the antirachitic effectiveness of sunshine varies with altitude, latitude, time of day, cloudiness and the amount of dust and moisture in the atmosphere. It has been shown also that there is a seasonal variation in the antirachitic effectiveness of sunshine coincident with the change in the inclination of the sun and with increased cloudiness in winter and increased skyshine in summer (Hess, '25; Hill, '27–'28; Mayer, '26; Russell, et al., '32; Tisdall and Brown, '27 a, b and '29). Since the antirachitic factor is required for reproduction, growth, maintenance of health and prevention of rickets, the character of the climate in any locality is of importance in determining to what extent sunshine can be relied upon as an antirachitic agent.

For this reason it was felt desirable to study the variation in the amount of antirachitically effective ultraviolet rays in sunshine at Ithaca, New York, and thus in the Great Lakes storm-belt area which possesses a climate closely comparable to Ithaca, since the amount of sunshine in this region is much

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less, particularly in the winter season, than in the remainder of the United States, except in the extreme Northwest.

The climatic and atmospheric conditions prevailing at Ithaca are as follows: latitude 42.5 degrees N., elevation 928 feet, average hours of sunshine 44 per cent, mean relative humidity 78 per cent, mean annual rainfall 32.9 inches, considerable snow and little dust or smoke.

When this study was started, little work had been done to determine the actual seasonal variation in the antirachitic effectiveness of sunshine in the different parts of the country, and no work had been reported wherein chicks were used as the experimental animal. Tisdall and Brown ('27 b), using the albino rat, showed that the antirachitic effect of sunshine at Toronto, Canada, in April and May is approximately eight times that in December, January and February. The rat, however, is not very sensitive to a lack of the antirachitic factor. Unless the diet contains a sub-optimum level of phosphorus and the calcium-phosphorus ratio is unduly widened, typical rickets cannot be developed in the rat. Chicks, on the other hand, are extremely sensitive. Even in the presence of a large excess of calcium and phosphorus, rickets, although somewhat delayed, invariably develops and in a severe form.

On account of this and also for certain practical considerations chicks were used as the experimental animal in the investigations reported here. The results obtained serve to check the results of Tisdall and Brown and show the relative value of the chick and the rat in rachitic studies.

The work of Bethke, Kennard and Kik ('25) and Heuser and Norris ('28–'29) shows that relatively small amounts of sunshine are needed to satisfy the antirachitic requirements of the growing chick. Furthermore, Russell and Massengale ('27–'28) and Scott, Hart and Halpin ('29–'30) have reported that sufficient antirachitically effective ultraviolet rays can be obtained from winter sunshine, at least in the regions studied, to prevent the development of rickets in chicks. None of these investigations, however, demonstrates the minimum

requirement of the chick during the different seasons of the year.

EXPERIMENTAL PROCEDURE

Because of the inclemency of the weather at Ithaca, it is not possible to expose chicks to direct sunshine during the late fall, winter and early spring. It was, therefore, necessary to make exposures behind a glass which transmits a known percentage of the antirachitic portion of the ultraviolet rays of sunshine. Corex-G980A was used, since this glass transmits practically all of the effective ultraviolet rays (Coblentz, '28) and is not subject to any appreciable amount of solarization (Coblentz, '29).

TABLE 1

Length of daily exposure of chicks to sunshine for each period

TREATMENT	ACTUAL DAILY EXPOSURES					
	Period 1 Dec. 22–Mar. 15 1928	Period 2 Mar. 22–June 14, 1928	Period 3 June 21–Sept. 13, 1928	Period 4 Nov. 9–Feb. 1, 1929	Period 5 Feb. 8–May 2, 1929	Period 6 May 10–Aug. 2, 1929
	Min.	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*[3]
Window glass	240	40	20	40	20	10
Corex-A glass	30	5	2½	5	2½	1¼
Corex-A glass	60	10	5	10	5	2½
Corex-A glass	120	20	10	20	10	5
Corex-A glass	240	40	20	40	20	10
Negative control	....	0	0	0	0	0
Light control	....	30[1]	30[2]	30[1]	30[1]	10[2]

[1] Carbon arc

[2] Direct sunshine (not exposed on rainy days)

[3] Exposures were made on alternate days for twice the time indicated, since it was impractical to attempt as short an exposure as 1¼ minutes.

Four exposing pens glazed with Corex-A and a control pen glazed with common window glass were used each season. In periods 2, 3, 4, 5 and 6 another control pen, receiving irradiation either from a twin carbon-arc lamp with 13 mm. 'therapeutic B' carbons at a distance of 36 inches from the floor or from direct sunshine, was included in the experimental work. Table 1 shows the exposures for the different periods.

All exposures were made at such a time that each period centered at high noon, the time when the sun is perpendicular to the meridian at Ithaca, 76.5°W.

The exposing pens were built on the south side of the brooding laboratory and were 4 feet square. The Corex glass covering the exposing pens was placed at an angle of 47.5° from the horizontal so that the sun's rays would be perpendicular to them at the spring and fall equinoxes. Approximately 13 square feet of glass were available for the transmission of light. The exposing pens were so constructed that the entering light was limited to that coming through the windows. The only other light received by the chicks was from 60 watt Mazda lamps. A standard 14-hour day was used in all seasons.

It was the original intention to arrange the experimental periods so that the summer periods would center at the summer solstice, the winter periods at the winter solstice and the spring periods at the spring equinox. However, it was impossible to start the first winter period until December 22nd. Consequently, the first three periods respectively follow rather than center at these dates. Since there was only 1 week between each of the 12-week periods, the fall period was omitted in 1928 in order that the winter period could start 6 weeks before the winter solstice. Limited time prevented the study of the fall period in 1929.

In all the periods, except the sixth, the chicks behind window glass and Corex-A were exposed daily regardless of the weather. The chicks in the sunshine control pens in periods 3 and 6 were not exposed on rainy days. In the sixth period it was thought impractical to attempt so short an exposure as 1.25 minutes. To make all pens comparable, the Corex-A, window glass and sunshine control pens were exposed on alternate days for twice the time given in tables 1 and 6.

Thirty day-old White Leghorn chicks of uniform breeding were put in each lot in all experiments. Each chick was weighed weekly and the weekly food consumption of each lot was recorded. At the time of weighing observations were

made of the rachitic condition of each chick as determined by the characteristic lameness. These were recorded by degrees of 1 to 4, 1 denoting slight lameness, 2 moderate lameness, 3 severe lameness and 4 extremely severe lameness. The weekly per cent degree of rachitic lameness was found by dividing the summation of the degrees by four times the number of birds present (four representing the theoretical maximum) and multiplying the result by 100. This measure was helpful in comparing the severity of rickets particularly in lots in which all the chicks were rachitic. Average weights were weighted for the influence of sex on growth.

TABLE 2

Composition and analyses of rations used

INGREDIENTS	PERIOD 1 (WINTER)	PERIOD 2 (SPRING)	PERIOD 3 (SUMMER)	PERIOD 4 (WINTER)	PERIOD 5 (SPRING)	PERIOD 6 (SUMMER)
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Ground yellow corn	52.5	52.5	57.0	57.5	57.5	57.5
Flour wheat middlings	20.0	20.0	20.0	20.0	20.0	20.0
Meat scrap (55 per cent protein)	10.0	10.0	....	....	....	....
Dried buttermilk	10.0	10.0	20.0	20.0	20.0	20.0
Alfalfa leaf meal	5.0	5.0	....	....	....	....
Steamed bone meal	2.0	2.0	2.5	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Per cent protein	17.3	17.3	14.9	14.9	14.6	15.0
Per cent calcium	1.968	2.210	1.185	0.901	0.897	0.955
Per cent phosphorus	1.186	1.316	0.985	0.815	0.777	0.774

Table 2 gives the percentage composition and analyses of the diets used in each of the periods. The diets used for the first three periods gave good growth, but did not produce sufficiently severe rickets at an early age and so were changed for the last three periods as indicated.

At the end of the sixth and twelfth weeks of periods 3, 4, 5 and 6 and at the end of the twelfth week of period 2, approximately 5 cc. of blood were taken by heart puncture (Sloan and Wilgus, Jr., '30–'31) from each of eight representative chicks per lot. Then the blood of each lot was pooled and

serum calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method and the serum inorganic phosphorus by the Fiske and Subbarow method as outlined by Hawk and Bergeim ('26).

At the end of the twelfth week of each period the left tibia and femur were taken from each of the eight chicks per lot from which the blood samples were drawn for the determination of bone ash. These bones were dried and freed of fat previous to ashing so that the per cent ash could be expressed on the moisture-free, fat-free basis.

During the last three periods an attempt was made to measure by chemical and physical means the relative amount of antirachitically effective ultraviolet rays present in sunshine. Of the various chemical methods available, the oxalic acid-uranyl sulfate method of Anderson and Robinson ('25) with slight modifications was chosen as being most suitable. Four quartz tubes containing oxalic acid were used. Two tubes were exposed directly to sunshine and the other two were exposed underneath common window glass. It was thought that by subtracting the grams of oxalic acid decomposed beneath the window glass from the grams of oxalic acid decomposed in the direct sunshine the decomposition due to the wave lengths shorter than 3130 Ängstrom units, that is, the waves having the greatest antirachitic effect, could be obtained.

The tubes were exposed on the south side of the analytical laboratory in a box constructed in such a manner as to avoid reflections from the box and also to prevent the freezing of the solutions. The box was mounted on hinges so that the tubes could be adjusted at right angles to the sun's rays during each exposure.

The apparatus used for the physical measurement of the effective ultraviolet rays was the Anderson and Gordon fluorescent ultraviolet photometer ('28) especially adapted for this purpose by Dr. H. P. Gage, of the Corning Glass Works, Corning, New York. This was accomplished by the use of a special uranium glass which fluoresces only when exposed

to ultraviolet rays shorter than 3130 Ängstrom units. The readings of the instrument give the amount of current in milliamperes necessary to bring a small light to the same brilliance as that caused by the fluorescence of the uranium glass from the incident ultraviolet rays of sunshine. These readings are then converted into intensity of antirachitic ultraviolet irradiation by means of a chart prepared by standardizing the instrument against a quartz mercury-vapor arc operated at 77 volts and 3.45 amperes. The intensity of the fluorescence of the uranium glass at 1 meter from the quartz mercury-vapor arc is called a 'Q' unit.

The results, therefore, are only an expression of the relative intensities of ultraviolet rays and not of the actual amount of ultraviolet-ray energy. To get the average intensity for 1 day or part thereof, a series of readings were taken at short intervals. These were made during the time the chicks were being exposed.

RESULTS

In view of the fact that the first three periods were somewhat of a preliminary nature and that the changes in the rations prevent the making of direct comparisons with the last three periods, it will suffice if only brief mention of the results obtained is made. These are summarized in table 3.

The results of period 1 (winter) indicated that an exposure of 1 hour or more through Corex-A[3] was sufficient to prevent the development of rickets. Although the average weight and bone ash were normal in the lot receiving 30 minutes' exposure, there was one chick recorded as being rachitic. This, considered with the results of studies of other glazing materials of lower transmissibility which accompanied this work, but are not reported here, seemed to indicate that 30 minutes' exposure was very close to the minimum.

The results of period 2 (spring) indicated that an exposure of 10 minutes or more was sufficient to prevent the develop-

[3] Hereafter in speaking of exposures to sunshine, exposures through Corex-A glass will be meant unless otherwise indicated.

ment of rickets. In the lot receiving an exposure of 5 minutes, there was one chick recorded as being rachitic, and the average weight, the grams of bone ash and the percentage of bone ash were slightly below the values for the other Corex and the carbon-arc lots. These results seem to justify the conclusion that an exposure of 5 minutes daily was slightly less than the minimum requirement. When compared with the results of the previous period, a winter to spring ratio of antirachitic effectiveness of sunshine of approximately 1:6 is indicated. A comparison of the sunshine record for these two periods with the 21-year average for these periods indicates that the ratio would have been somewhat narrower in a normal season (table 7).

At the end of period 3 (summer) there were no external symptoms of rickets in any of the lots except the negative controls. However, in the lot receiving the 2.5 minute exposure, the average weight, the grams of bone ash and percentage of bone ash were slightly below normal. Considering these results and those of the studies of other glazing materials previously mentioned, it seems that the exposure of 2.5 minutes daily was slightly less than the minimum protective exposure. Due to the change in ration, this period cannot be compared directly with the two previous periods, but the results indicate roughly a ratio of effectiveness of spring and summer sunshine of approximately 1:2.

A comparison of the increase in hours of sunshine during the periods of exposure for these three periods with the increased antirachitic effectiveness, indicates quite clearly that the increase in effectiveness from winter to spring was due very largely to an increase in intensity, since there was an increase of only 31.6 per cent in the hours of sunshine between 11 A.M. and 1 P.M. The increase in effectiveness from spring to summer was apparently due both to an increase in hours of sunshine as well as to an increased intensity.

A minimum exposure of 5 minutes and a maximum of 40 minutes daily were used in period 4 (winter), since the results of the previous winter period indicated that the length of ex-

TABLE 3

Summary of results of 12-week-old chicks, periods 1, 2, 3

GLAZING MATERIAL	MINUTES' EXPOSURE	PERIOD 1 (WINTER)			PERIOD 2 (SPRING)			PERIOD 3 (SUMMER)		
		Average weight in grams	Grams bone ash	Per cent bone ash	Average weight in grams	Grams bone ash	Per cent bone ash	Average weight in grams	Grams bone ash	Per cent bone ash
Window glass	240,40,20	708	2.16	43.7	530	1.58	41.3	392	1.11	42.7
Corex-A glass	2.5	....	....	....	....	....	....	778	2.20	47.2
Corex-A glass	5	....	....	....	768	2.43	44.6	852	2.48	48.3
Corex-A glass	10	....	....	....	847	2.78	46.3	773	2.38	48.7
Corex-A glass	20	....	....	....	852	2.75	46.7	817	2.63	49.3
Corex-A glass	30	889	2.90	48.9	....	....	....	....	....	....
Corex-A glass	40	....	....	....	810	2.80	45.9	....	....	....
Corex-A glass	60	861	2.78	49.3	....	....	....	....	....	....
Corex-A glass	120	866	2.88	48.0	....	....	....	....	....	....
Corex-A glass	240	914	2.89	47.9	....	....	....	....	....	....
Negative control	0	....	....	....	515	1.54	41.3	402	1.12	41.9
Light control	30	....	....	....	838	2.71	45.8	844	2.66	49.0

TABLE 4

Summary of results of 12-week-old chicks; period 4, winter; November 9, 1928, to February 1, 1929

GLAZING MATERIAL	MINUTES' EXPOSURE	AVERAGE WEIGHT IN GRAMS	MILLIGRAMS Ca PER 100 CC. BL. SERUM	MILLIGRAMS P PER 100 CC. BL. SERUM	PER CENT RICKETS	PER CENT DEGREE RICKETS	GRAMS BONE ASH	PER CENT BONE ASH
Window glass	40	275.5	8.80	5.90	100.0	98.2	0.766	37.76
Corex-A glass	5	402.4	7.70	6.18	100.0	95.0	1.137	37.62
Corex-A glass	10	516.8	9.92	4.96	90.0	73.8	1.469	38.96
Corex-A glass	20	708.6	9.95	5.68	38.5	23.1	1.975	42.18
Corex-A glass	40	796.9	10.94	5.58	10.7	6.3	2.429	45.53
Negative control	0	263.6	8.54	5.14	100.0	100.0	0.748	36.48
Light control [1]	30	869.6	11.77	5.82	0.0	0.0	2.619	47.35

[1] Carbon arc

posure would have to be reduced to get below the minimum requirement.

A comparison of the results of the 40-minute lot with those of the carbon-arc control (table 4) showed slight but quite evident indications of rickets. The average weight and grams and percentage of bone ash were slightly below the carbon-arc control. Moreover, there were three chicks in this lot with characteristic rachitic lameness, although only one case was severe. The external symptoms of rickets did not appear until the eighth week. These results indicate that, although 40 minutes was not sufficient to entirely prevent rickets, it was close to the minimum requirement.

The number of hours of sunshine for this period was 13.7 per cent greater than the 21-year average. Therefore, for an average season the minimum exposure required would probably be in the neighborhood of 45 minutes.

The results of the fifth period (table 5) indicate that there was considerably more effective ultraviolet rays in this period than in the previous winter period. Although the average weight, serum calcium and percentage of bone ash of the 10-minute lot were normal, there were two chicks at the end of the 12 weeks with slight but definite external symptoms of rickets. One chick died during the eighth week with severe rickets and one of the chicks taken for blood and bone analysis showed slight beading of the ribs. This chick and the one that died were recorded as being moderately rachitic after the fourth week. This shows that 10 minutes' daily exposure was nearly but not completely protective.

The condition of rickets in the 10-minute lot in this period was quite similar to that in the 40-minute lot, period 4. Thus it took approximately four times the exposure in the winter to produce the same results as were obtained with 10 minutes in the spring. This gives an approximate ratio of effectiveness for winter as compared to spring of 1:4, a ratio which is somewhat narrower than was found for the previous winter and spring periods (period 1 and 2).

Periods 1 and 2 are not strictly comparable, respectively, with periods 4 and 5, since the diet used for the latter two periods contained less calcium and phosphorus and the periods of exposure were 6 weeks earlier. However, the ratio of the minimum protective exposures for periods 4 and 5 should be

TABLE 5

Summary of results of 12-day-old chicks; period 5, spring; February 8 to May 2, 1929

GLAZING MATERIAL	MINUTES' EXPOSURE	AVERAGE WEIGHT IN GRAMS	MILLIGRAMS Ca PER 100 CC. BL. SERUM	MILLIGRAMS P PER 100 CC. BL. SERUM	PER CENT RICKETS	PER CENT DEGREE RICKETS	GRAMS BONE ASH	PER CENT BONE ASH
Window glass	20	318.7	9.27	6.72	100.0	92.6	0.852	42.85
Corex-A glass	2½	475.4	10.10	6.38	74.1	48.1	1.353	[2]
Corex-A glass	5	666.9	11.54	6.74	40.7	20.4	1.982	[2]
Corex-A glass	10	841.0	12.65	6.00	7.4	2.8	2.841	50.39[3]
Corex-A glass	20	879.4	12.89	5.38	0.0	0.0	2.590	50.68
Negative control	0	308.6	8.83	6.14	100.0	91.2	0.804	40.14
Light control [1]	30	847.1	14.44	6.38	0.0	0.0	2.503	50.48

[1] Carbon arc

[2] Ash determinations lost

[3] Average of five bones; other three lost

TABLE 6

Summary of results of 12-day-old-chicks; period 6, summer; May 10 to August 2, 1929

GLAZING MATERIAL	MINUTES' EXPOSURE	AVERAGE WEIGHT IN GRAMS	MILLIGRAMS Ca PER 100 CC. BL. SERUM	MILLIGRAMS P PER 100 CC. BL. SERUM	PER CENT RICKETS	PER CENT DEGREE RICKETS	GRAMS BONE ASH	PER CENT BONE ASH
Window glass	10	360.2	7.89	6.88	100.0	87.5	0.942	36.67
Corex-A glass	1½	615.1	9.78	6.52	62.5	34.4	1.675	41.29
Corex-A glass	2½	626.3	9.86	6.64	34.6	24.0	1.660	41.70
Corex-A glass	5	819.0	10.66	7.12	3.7	0.9	2.383	46.37
Corex-A glass	10	742.0	11.28	7.40	0.0	0.0	2.139	45.56
Negative control	0	390.7	7.74	5.64	100.0	92.1	1.020	35.96
Light control [1]	10	818.1	11.12	6.56	0.0	0.0	2.421	44.03

[1] Direct sunshine

narrower, due to the fact that the amount of sunshine during the hours of 11 A.M. to 1 P.M. in period 5 (spring) was only 10 per cent greater than for period 4 (winter), whereas for period 2 (spring) the amount of sunshine during this time was 31.6 per cent greater than for period 1 (winter). Had there been an increase in the amount of sunshine from winter to spring in periods 4 and 5 comparable to that in periods 1 and 2, it seems probable that the ratio of effectiveness of sunshine from winter to spring, would have been closer to the ratio of 1:6 found for periods 1 and 2. Upon consideration of this and of the 21-year average, it seems probable that the ratio of effectiveness of an average season is slightly narrower than 1:6. Since the increase in the amount of sunshine between the hours of 11 A.M to 1 P.M. for these periods was not very great, it is evident that the increase in effectiveness of spring sunshine over winter sunshine was due almost entirely to an increase in intensity of ultraviolet rays.

Since an exposure of 2.5 minutes was nearly protective during the previous summer period, the shortest exposure was reduced to 1.25 minutes for period 6. This reduction with the decrease in the calcium and phosphorus content of the diet proved to be sufficient to make the exposure of 1.25 minutes sub-minimal (table 6).

When compared with the sunshine control pen which received 10 minutes' daily exposure to direct sunshine, the chicks which received 5 minutes' daily exposure through Corex-A glass seemed to be nearly normal. The average weight of the latter lot was practically the same as the weight of the sunshine control lot. The percentage of bone ash, however, was slightly lower in the 5-minute Corex lot than that of the sunshine control lot. The serum calcium was also lower in the 5-minute lot than that of the sunshine control lot. These differences do not point conclusively toward a rachitic condition in the 5-minute lot, but, since they are consistent and since there was one chick in this lot which showed slight rachitic lameness at the end of the twelfth week, it seems reasonable to conclude that there was a condition of slight rickets present.

The low average weight of the chicks in the lot receiving 10 minutes' exposure cannot be accounted for. The grams and percentage of bone ash were somewhat lower than in the sunshine control lot, but this is not considered to be conclusive evidence of rickets, since the serum calcium was normal and the lot receiving half the exposure was nearly normal in all respects. There were no external or internal symptoms of rickets.

A comparison of the results of periods 5 and 6 shows that approximately the same degree of rickets prevailed in the 5-minute lot in the summer as in the 10-minute lot in the preceding spring. This shows that about twice the exposure was required in the spring period as for the summer period, making a spring-summer ratio of effectiveness of 1:2.

There was a 55.4 per cent increase in the amount of sunshine in period 6 during the hours of 11 A.M. to 1 P.M. as compared to period 5. This indicates that the increased effectiveness for this period (summer) was due both to an increase in amount of sunshine and to an increase in intensity.

As the condition of rickets in the 40-minute Corex lot in period 4 (winter) was similar to that in the 5-minute Corex lot in period 6 (summer), the exposure required during winter to prevent the development of rickets was about eight times as great as was required for the summer, giving a ratio of effectiveness of 1:8.

In view of the fact that there is some relation between the quantity of sunshine between 11 A.M. and 1 P.M. and the total hours of sunshine, the probable ratio for an average year was calculated. These calculations were based upon the variations in total hours of sunshine of the years 1928 and 1929 from the 21-year average (table 7). The results showed that the calculated winter-spring-summer ratio of effectiveness for an average year, periods 1, 2 and 3, was 1:5.3:9.3 and that of periods 4, 5 and 6 was 1:5.9:8.1. In view of all the variables concerned, this is remarkably close agreement for experimental work of this character. The conclusion seems justified, therefore, that the antirachitic effectiveness of sunshine for the

winter, spring and summer seasons varies in accordance with the ratio 1 : 5-6 : 8-9.5.

The increase in effectiveness of sunshine from winter to summer of approximately eight to nine times is in accord with the data of Dorno (Hess, '25), showing that the intensity of the ultraviolet rays of sunshine increased about 8.75 times from January 15th to July 15th. On the other hand, the increase in effectiveness of sunshine from winter to spring was not found to be as great as was found by Tisdall and Brown ('27), who concluded that the antirachitic effect of sunshine in April and May was about eight times as great as in December, January and February. Since Toronto, Canada, is also in the Great Lakes storm belt, it is logical to conclude that the slight difference in latitude would not affect the results. It is more probable, therefore, that the difference in results can be ascribed to the difference in the amount of sunshine, since the amount recorded by Tisdall and Brown at Toronto for the 2-hour period, 11 A.M. to 1 P.M., for the months of December, January and most of February, 1926–1927, is less than that recorded for the same period at Ithaca in 1928–1929, while the amount for their 4-week experimental period ending May 9, 1927, at Toronto was slightly greater than that for the same period at Ithaca in 1929.

TABLE 7

Total hours and percentage of possible sunshine at Ithaca, New York[1]

PERIOD	21-YEAR AVERAGE SUNRISE TO SUNSET		1928–29 SUNRISE TO SUNSET		1928–29 11 A.M. TO 1 P.M.		HOURS 1928–29 ± 21-YEAR AVERAGE
	hours	*per cent*	*hours*	*per cent*	*hours*	*per cent*	*per cent*
1. (Jan.–Mar.)	373.3	38	319.4	33	89.6	49	—14.4
2. (Apr.–June)	679.4	52	658.0	50	117.9	65	— 3.1
3. (July–Sept.)	731.8	58	805.1	63	147.9	80	+10.0
4. (Nov.–Jan.)	230.8	26	262.5	30	76.8	42	+13.7
5. (Feb.–Apr.)	465.2	43	361.0	33	84.5	47	—22.4
6. (May–July)	775.7	57	867.9	63	131.2	71	+11.9

[1] Records of the Ithaca Station of the United Weather Bureau

The possibility that the difference in results was due in part to the use by Tisdall and Brown of the rat as the experimental animal and of a rachitogenic diet in which the quantity of calcium and phosphorus was greatly unbalanced was not revealed by the evidence obtained. Both types of experimental animals on the respective rachitogenic diets used appeared equally sensitive to a lack of the effective ultraviolet rays of sunshine.

These experiments demonstrate quite clearly the small amount of sunshine required even in the winter time, to prevent the occurrence of rickets in chicks. Since only a small part of the United States is in a region of as little average sunshine (44 per cent) as Ithaca, New York (Ward, '25), the conclusion seems justified that sunshine can quite generally be depended on as a source of the antirachitic factor for chicks, if the chicks are exposed directly or behind ultraviolet transmitting materials, the period of exposure depending on the percentage transmission of the material used. In view of the fact that 30 minutes was found to be protective in period 1 during the winter, there are probably three to four times as much antirachitically effective ultraviolet rays available as are necessary for chicks, since it has been shown that in this season the effective ultraviolet rays are at their greatest intensity between the hours of 11 A.M. and 12 M. and that the intensity from 10 A.M. until 11 A.M. and from 12 M. to 1 P.M. is nearly as great (Hess, '25).

It should be remembered that the minimum exposures found were obtained behind glass which transmits a maximum of 92 per cent due to the reflection from the surface (Coblentz, '28). The absolute minimum exposures required without glass, therefore, would be 8 per cent less. This fact is important in estimating minimum exposures under conditions where it would not be necessary to use glass, but it would probably not change the ratio of minimum exposures required in the different seasons.

The relatively high susceptibility of chicks to rickets makes the results of this work very applicable to other types of ani-

mals requiring the antirachitic factor for proper development and maintenance of health and to human beings. Unfortunately, there is no definite information to indicate whether or not humans have a higher requirement for the antirachitic factor than chicks. However, because of the possibility for a greater body-surface exposure, it is probable that where a high-transmitting glazing material is used, sufficient beneficial effect can be obtained in most parts of the United States even in the winter time from the use of the available sunshine as a source of the antirachitic factor even though the period of exposure is limited to a short time during the middle of the day.

TABLE 8

Amount of sunshine during periods 4, 5, and 6 expressed in milligrams of oxalic acid decomposed and in 'Q' units

PERIOD	AVERAGE AMOUNT OXALIC ACID DECOMPOSED DAILY				AVERAGE 'Q' UNITS
	Exposure	Direct sunshine	Window-glass filter	Difference	
	min.	*mg.*	*mg.*	*mg.*	
4 (winter)	40	22.19	16.69	2.50	0.618
5 (spring)	10	8.25	7.13	1.12	1.150
6 (summer)	5	6.31	5.44	0.87	3.481

It is probable, moreover, that people working constantly out-of-doors in the winter time have no need for other sources of the antirachitic factor, even though only the face and occasionally the hands are exposed to the available ultraviolet rays. This observation is supported by the fact that in chicks protection against the development of rickets depends upon the exposure of head parts and feet (Maughan, '30), as down and feathers screen out the effective ultraviolet rays the same as the clothing of human beings.

The results of the chemical and physical studies (table 8) did not show the same change in the antirachitic effectiveness of sunshine as did the work with chicks. According to the chemical method, there was an average of 2.5 mg. of oxalic acid decomposed daily during an exposure of 40 minutes by the ultraviolet rays not transmitted by window glass in

period 4 (winter, 1928–1929). In period 5 (spring) the average daily decomposition of oxalic acid was 1.12 mg. during a 10-minute exposure and for period 6 (summer) 0.87 mg. during a 5-minute exposure. If this method had shown the same relationship between the seasons as did the work with chicks, the decomposition of oxalic acid would have been the same for the three periods, since the length of the minimum protective exposures for the chicks during the winter, spring and summer were, respectively, 40, 10 and 5 minutes. The results obtained by this method, therefore, did not show the true relationship of the amount of antirachitically effective ultraviolet energy received by the chicks during periods 4, 5 and 6.

The results obtained by physical measurement approximate the results obtained with chicks more closely. The average daily 'Q' units for the winter, spring and summer periods were, respectively, 0.618, 1.150 and 3.481. As the readings with this instrument are instantaneous, the average 'Q' units would be expected to be greater for the spring than for winter and greater for the summer than for spring. There was a considerable seasonal increase in the readings as is shown by the above values. These give a ratio for the three seasons, winter, spring and summer, of 1 : 1.86 : 5.63. This method indicated somewhat more accurately the amount of antirachitically effective ultraviolet rays incident in the three seasons than the other method used, but it does not appear sufficiently reliable to replace biological studies.

SUMMARY

1. The minimum daily exposure to sunshine required to prevent the development of rickets in chicks at Ithaca, New York, in 1927–1928 appeared to be approximately 30 minutes during winter, 5 minutes during spring and 2.5 minutes during summer, when the exposures were made behind glass which transmitted practically all the ultraviolet rays of sunshine, except those lost by reflection and when the 12 weeks' experimental period used began at the December and June solstices and the March equinox. With an experimental period of equal

length, but centering rather than beginning at these dates and using a rachitogenic diet possessing a smaller quantity of calcium and phosphorus, the minimum protective exposure in 1928–1929 was found to be approximately 40 minutes during the winter, 10 minutes during the spring and 5 minutes during the summer.

2. When the results obtained in 1927–1928 were calculated in terms of the 21-year average of sunshine, it was found that the winter-spring-summer ratio of effectiveness of an average year was 1 : 5.3 : 9.3. The ratio of an average year based upon the 1928–1929 results was found to be 1 : 5.9 : 8.1. It appears, therefore, that the antirachitic effectiveness of sunshine for the winter, spring and summer seasons at Ithaca, New York, varies in accordance with the ratio 1 : 5-6 : 8-9.5. Since Ithaca, New York, is in one of the two areas in the United States with least amount of sunshine, it is probable that the results obtained in this experimental work are an expression of the minimum antirachitic effectiveness of sunshine in the United States rather than an expression of the average or maximum effect.

3. The increase in antirachitic effectiveness of spring sunshine at Ithaca as compared to winter sunshine was caused largely by an increase in intensity rather than in the amount of sunshine. On the other hand, the increase in effectiveness of summer sunshine as compared to spring sunshine appeared to be due about equally to both increase in intensity and increase in amount of sunshine.

4. The quantity of antirachitically effective ultraviolet rays of sunshine was not accurately shown either by a modification of the oxalic acid-uranyl sulfate method of Anderson and Robinson or by an adaptation of the Anderson and Gordon fluorescent ultraviolet photometer.

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